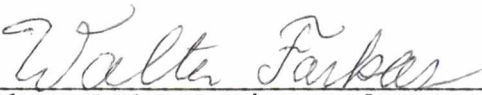


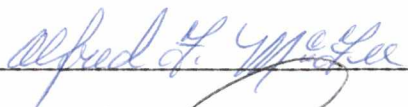
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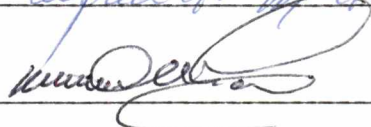
I am submitting herewith a thesis written by Teresa L. Rusk entitled "Nephrotoxic Effects on the Offspring of Rats Chronically Treated with Mercuric Chloride." I recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Life Sciences.



Walter Farkas, Major Professor

We have read this thesis
and recommend its acceptance:





Accepted for the Council:



Vice Chancellor
Graduate Studies and Research

NEPHROTOXIC EFFECTS ON OFFSPRING OF RATS
CHRONICALLY TREATED WITH MERCURIC CHLORIDE

A Thesis
Presented for the
Master of Science
Degree
The University of Tennessee, Knoxville

Teresa L. Rusk

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ABSTRACT

Repeated subcutaneous injections of HgCl_2 at a dose of either 4.0 or 2.0 mg/kg produced toxic effects in rats when administered during the last 9 days of gestation. Females exhibited diarrhea, anorexia and weight loss of varying severity. Maternal renal function was monitored by urinalysis and was shown to be impaired during the early days of treatment but returned to normal later. Histological evaluation of adult kidneys after 2 days of treatment demonstrated the nephrotoxic effects of HgCl_2 ; however, after 9 or 19 HgCl_2 injections, kidneys showed little evidence of damage. The adult kidneys were apparently capable of repair and regeneration in spite of continued administration of the nephrotoxic agent. Evidence of the regenerative process was found in tubules lined with densely packed, flattened cuboidal epithelial cells. These cells did not stain for mitochondria, and were probably not functionally mature. As indicated by the normal urinalysis results, these immature tubular cells did not result in a functional deficit, demonstrating the great functional reserve of the kidney.

Administration of HgCl_2 to pregnant rats was not overtly toxic to their developing offspring, perhaps because of the time of administration. Pup birth weight among HgCl_2 -exposed litters, however, was significantly lower than that of control litters. Nephrotoxic effects were not identifiable in pups at birth or 12 days later, though the presence of mercury was confirmed in fetal kidneys after 2 days of exposure. Effects could have been too subtle to detect by light microscopy, or nephrotoxic injury may have occurred but been masked by

subsequent renal development. If the latter is true, it would appear that the perinatal rat kidney, by the very nature of its development, is resistant to HgCl_2 -induced injury and thus may not be as sensitive to mercury intoxication as its adult counterpart or as other developing tissues.

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CHAPTER 1

INTRODUCTION

Many environmental contaminants such as paraquat, dioxin and polychlorinated biphenyls have been shown to be nephrotoxic in a variety of animal species. The nephrotoxicity of several drugs, most commonly analgesics and aminoglycoside antibiotics, has been demonstrated, and a variety of other chemical nephrotoxins, such as diphenylamine, chloroform and carbon tetrachloride, have been identified. The heavy metals, including mercury, constitute one of the major classes of known nephrotoxins. The nephrotoxicity of most of these agents, however, has not yet been evaluated in the fetal or neonatal animal.

Mercury has been used extensively as a model compound to produce renal failure in experimental animals. Both organic and inorganic forms cause selective necrosis of the proximal tubules, which may, at small doses, produce signs of proteinuria, glucosuria and polyuria and at larger doses lead to anuria and death.

Despite the considerable amount of data that has accumulated on mercury nephropathy in adults, very little information is available concerning the effects of prenatal exposure to mercury on renal development. Inasmuch as the adult kidney can be affected by mercury, it is expected that the developing kidney may also be vulnerable to damage at various stages of development, both pre- and postnatal.

Many case reports and experimental studies have shown teratogenic and embryocidal effects of mercury at levels which did not produce discernible maternal toxicity. Since mercury appears to pose a greater

danger to developing tissues in general than to adult tissues, the immature kidney may in fact be more sensitive to mercury than its adult counterpart.

Due to the widespread occurrence of mercury, the pregnant animal has great opportunity for exposure. Industrial and agricultural uses, as well as the combustion of coal and petroleum products, release substantial quantities of mercury into the environment. Moreover, mercury in the environment is often bioconcentrated and thus can enter the food chain in significant quantities.

Given the widespread occurrence of mercury and its well-documented nephrotoxic effects in adults and teratogenic effects in their offspring, it would appear that the potential risk to the developing animal is great.

This research was designed to determine whether perinatal exposure to mercury would induce identifiable changes in the morphology of the developing rat kidney. Neonatal kidneys were examined at birth and 12 days later in an attempt to evaluate nephrotoxic effects of both transplacental mercury exposure and exposure via lactation. In this way, the effects of mercury on both pre- and postnatal development were assessed. Maternal renal function was monitored during treatment and renal morphology was examined to allow some comparison of the relative effects of mercury on the mature kidney and on the developing organ.

CHAPTER 2

REVIEW OF LITERATURE

Mercury is a widespread environmental pollutant which poses a threat to human health via contamination of water and food resources. Although significant concentrations of mercury and other metals exist in the air, soil and water as natural deposits, technologic use of these non-biodegradable materials has led to their accumulation in the environment (Hammond and Beliles, 1980).

The largest uses of mercury at present are industrial, including the electrolyte production of chlorine and caustic soda and the manufacture of electrical equipment and anti-fouling paint. Agricultural products, such as pesticides and seed treatments, account for the use of substantial quantities of mercury. Crop applications, discharges from industrial plants and exhausts from metal smelters return to the environment as much as a third of the mercury used. A significant amount of mercury also enters the environment through the combustion of coal and petroleum products (Hammond, 1971).

Metals released into the environment are often bioconcentrated and enter the food chain. It is in this way that mercury poses its greatest threat to human health. Though mercury poisoning occurs to some extent among certain occupational groups (primarily from inhalation of inorganic mercury vapor), more important have been the recent episodes of widespread poisoning due to environmental contamination (Hammond and Beliles, 1980). The bioconcentration in fish and shellfish of methylmercury from industrial plants in Minamata Bay, Japan proved to be

responsible for an outbreak of mercury poisoning in local residents who consumed the contaminated fish (Hammond and Beliles, 1980). The clinical syndrome known as "Minamata disease" is characterized primarily by central nervous system disturbances such as ataxia, tremor, progressive incoordination, impairment of vision, speech and hearing, and intellectual deterioration (Chang, 1977). At the time of these outbreaks of Minamata disease, effects on other organ systems were apparently not evaluated or reported, perhaps because of the predominance of neurological symptoms. Another, more extensive outbreak of Minamata disease occurred in Iraq; it involved some 6000 cases and 500 deaths among people who had consumed bread made from mercury-treated grains (Bakir et al., 1973).

Placental transfer of mercury has been implicated in the etiology of "congenital" Minamata disease. Six percent of infants born in the area of Minamata Bay during the period 1954-1959 were affected with a cerebral palsy-like disease thought to be the result of mercury poisoning. Interestingly, the affected infants had never eaten contaminated fish or shellfish, though their mothers had during pregnancy but showed no apparent effects. These infants had abnormally high levels of mercury in their hair, and those who died showed high levels of mercury in brain, liver and kidney tissues at autopsy (Nelson et al., 1971). Children born with congenital Minamata disease manifest a variety of psychomotor disturbances and various degrees of mental retardation (Reuhl et al., 1981a).

Both organic and inorganic forms of mercury have been shown to cross the placenta and thus be transferred to developing offspring

(Berlin and Ullberg, 1963; Matsumoto et al., 1965; Suzuki et al., 1967; Suzuki et al., 1971; Khera and Tabacova, 1973; Garcia et al., 1974; Spyker and Chang, 1974; Chang and Sprecher, 1976; Su and Okita, 1976; Chang et al., 1977a; Chang et al., 1977b; Reuhl et al., 1981a,b). In a comparison of mercury contents in maternal blood, umbilical cord blood and placental tissues of normal Japanese women at delivery, Suzuki et al. (1971) found the highest levels of mercury in the placentas, suggesting that perhaps the metal accumulates there.

Postnatal exposure to mercury via lactation may also contribute to the etiology of the infantile form of Minamata disease and other cases of neonatal mercury intoxication (Nelson et al., 1971). Berlin and Ullberg (1963) have demonstrated the occurrence of mercury in mammary glands of mice. Other investigators (Bakulina, 1968; Deshimaru, 1969; Yang et al., 1972b; Garcia et al., 1974) have demonstrated infantile mercury intoxication as a result of exposure through their mothers' contaminated milk. By feeding tracer doses of mercury to rats, Garcia et al. (1974) showed that pups exposed prenatally then nursed by untreated foster dams generally had lower tissue mercury levels than did pups exposed prenatally then reared by the same treated dams.

Since the outbreak of mercury poisoning in Minamata disease, much of the research on mercury poisoning in both adults and neonates has been concerned with mercury's toxic effects on the central nervous system (Miyakawa and Deshimaru, 1969; Chang and Hartman, 1972a,b,c; Klein et al., 1972; Chang et al., 1974; Somjen et al., 1974; Spyker and Chang, 1974; Fehling et al., 1975; Chang et al., 1977a; Chang et al., 1977b; Reuhl et al., 1981a,b). Though in many cases neurological

disturbances dominate the clinical picture of mercury intoxication (Chang, 1977), the kidney, in almost all circumstances, accumulates the highest concentration of mercury (Clarkson, 1972; Iverson et al., 1973; Ware et al., 1974a,b; Hammond and Beliles, 1980) and is also susceptible to mercury toxicity.

Mercury is a potent nephrotoxin, as are most heavy metals, and relatively small doses may produce signs of glucosuria, proteinuria, aminoaciduria and polyuria. Increased doses can lead to renal tubular necrosis, anuria, elevated blood urea nitrogen levels and death (Hook, 1980). Mercury rapidly and preferentially accumulates in the renal cortex (Berlin and Ullberg, 1963; Hook, 1980), rendering cells of the proximal tubules particularly susceptible to the toxic properties of mercury (Fowler, 1974). Both organic and inorganic forms have been shown to cause selective necrosis of the proximal tubules (Rodin and Crowson, 1962a,b; Taylor, 1965; Gritzka and Trump, 1968; Fowler, 1972; Klein et al., 1973; Ware et al., 1973; Fowler, 1974; Ganote et al., 1975; Ware et al., 1975). Damage is primarily to the terminal segment of the proximal tubule, the pars recta (Rodin and Crowson, 1962a; Verity and Brown, 1970; Fowler, 1972; Ganote et al., 1975; Ware et al., 1975), though larger doses of mercury have been shown to cause a more widespread necrosis (Simonds and Hepler, 1945).

Inorganic mercury causes epithelial necrosis of renal tubules with persistence of the basement membrane (Biber et al., 1968). Loss of brush border, cytoplasmic degradation and cell exfoliation are frequent observations in cases of inorganic mercury nephropathy (Prescott and Ansari, 1969; Ware et al., 1973; Ganote et al., 1975; Ware et al., 1975;

Kempson et al., 1977). Mitochondrial changes, including swelling and transformations, are often detected with electron microscopy (Gritzka and Trump, 1968; Ware et al., 1973; Ganote et al., 1975; Ware et al., 1975). Organic mercury poisoning induces lesions of a similar nature, including swollen, vacuolated epithelial cells, focal cytoplasmic degradation and mitochondrial changes (Klein et al., 1973; Ware et al., 1973, 1975; Stroo and Hook, 1977).

The kidney has a great functional reserve which often enables it to overcome acute nephrotoxic insult (Hook, 1980). In response to the damage to part of the proximal tubule as a result of mercury, Biber et al. (1968) found structural and functional evidence of adaptive compensation in the portion of the proximal tubule that had escaped the most severe injury. A phase of repair and regeneration follows the acute, necrotic phase of mercury-induced tubular injury (Oliver, 1953; Cuppage and Tate, 1967; Biber et al., 1968; Kramp et al., 1974). The intact basement membrane serves as a guiding surface for the proliferation of new epithelial cells which arise from those that have escaped the toxic insult (Oliver, 1953).

Cuppage and Tate (1967) have described the regenerative phase of mercury nephropathy in rats. Within 3 days after a single injection of mercury, numerous flattened, early regenerating cells, were observed along the denuded basement membrane in the necrotic zone. Mitotic figures were frequently seen in the regenerating cells. By 5 days post-injection, the entire tubule was relined by low cuboidal cells. These cells contained relatively few organelles, such as mitochondria and lysosomes. Eventually the newly relined tubules were

indistinguishable from normal ones. After a period of maturation, the new epithelium was morphologically and enzymatically similar to that of the original tubular lining, and thus capable of normal function (Oliver, 1953; Cuppage and Tate, 1967).

Mercury appears to pose a greater danger to developing tissues in general than to adult tissues (Hammond, 1971). The teratogenicity of mercury has been demonstrated in experimental animals (Ramel, 1967; Khera and Tabacova, 1973; Gilani, 1975; Su and Okita, 1976; Fuyuta et al., 1978; Chen et al., 1979), and many cases of congenital damage have been reported in infants born to mothers who were exposed to mercury during pregnancy, but who were themselves apparently not harmed (Nelson et al., 1971; Snyder, 1971; Choi et al., 1978). Administration of relatively high levels to pregnant animals causes fetal death or teratogenic injury (e.g., cleft palate, missing limbs and facial deformities), frequently without discernible maternal toxicity (Harris et al., 1972; Spyker and Smithberg, 1972; Khera, 1973; Khera and Tabacova, 1973; Su and Okita, 1976; Fuyuta et al., 1978; Chen et al., 1979).

Degenerative changes similar to those observed in adult kidneys have been described in the neonatal kidney following transplacental exposure to small amounts of methylmercury (Chang and Sprecher, 1976). As in the adult, mercury primarily affected the proximal tubules of the fetal kidneys. Edema of epithelial cells, accumulation of lysosomes and enlargement of the apical vacuoles were observed by electron microscopy. Unlike the adult situation however, degenerative changes in the glomeruli of the neonatal kidneys were also observed, including

degeneration of the epithelial cells of Bowman's capsule, fusion of the glomerular epithelial foot-processes and detachment of the glomerular endothelial cells from their basement membranes. These findings suggest that the developing kidney may be more sensitive to the toxic effects of mercury than its adult counterpart.

Khera and Tabacova (1973) found that oral administration of methylmercuric chloride at 5 mg/kg/day to pregnant mice on days 6 through 17 resulted in 100% stillbirths or neonatal deaths with no apparent toxicity to the dams. Su and Okita (1976) demonstrated embryocidal and teratogenic effects of mercury in three strains of mice. At non-embryocidal levels, cleft palate was the most frequently observed malformation in the pups, and the incidence of maternal toxicity was low. Cleft palate was the most frequently seen malformation in rat pups also (Fuyuta et al., 1978). Neurotoxic signs in the dams were apparent only at high, embryocidal doses. Chen et al. (1979) found that administration of low doses of mercury throughout gestation resulted in smaller offspring without anatomical abnormalities, but no signs of maternal toxicity.

Even very small amounts of mercury administered during pregnancy (2.0-4.0 mg/kg body weight), while not producing maternal toxicity or gross fetal defects, have been shown to interfere with the normal fetal development of the brain (Chang et al., 1977a; Chang et al., 1977b; Reuhl et al., 1981a,b), liver (Ware et al., 1974) and kidney (Chang and Sprecher, 1976).

It is possible that the greater risk for mercury to the newborn may be due to the facilitated placental transfer and increased fetal tissue

binding of mercury (Chang et al., 1977b). Fetal tissues seem to have a high affinity for mercury (Chang et al., 1977a) and studies have shown that mercury concentrates in high levels within the developing embryo (Berlin and Ullberg, 1963; Matsumoto et al., 1965; Suzuki et al., 1967; Yang et al., 1972a; King et al., 1976). Tejining (1967) demonstrated a preferential concentration of methylmercury in the human fetus over the maternal tissues and increased fetal tissue binding of mercury has been shown in experimental animals as well. After exposure of pregnant mice to mercury, Suzuki et al. (1967) found a 30% higher concentration of the metal in fetal erythrocytes than in maternal ones. Exposure of pregnant rats to a single dose of methylmercury resulted in a four-fold greater mercury concentration in the fetal brain than in that of the mother (Matsumoto et al., 1967). King et al. (1976) fed tracer doses of mercury to pregnant rats and found that, based on dry weight comparisons, the fetal brain concentration of mercury was 3.4 times higher than the maternal brain concentration. The partitioning fraction (amount of mercury in the organ/total body burden of mercury) of the fetal brain was 19 times greater than the partitioning fraction of the maternal brain.

Despite their findings that only about 1% of the dose fed to the dam is transferred to the pup during a 21-day lactation period, Yang et al. (1972b) showed that nursing rat pups accumulated higher concentrations of mercury in their brains than is found in their mother's brains. They proposed that neonatal brain tissue has either a greater affinity or a longer biological half-life for mercury than does maternal brain tissue.

It has been shown in adults that the kidneys retain mercury longer than other tissues so that with increasing time after exposure, a greater proportion of the body burden is found within the kidneys (Clarkson, 1972). It could be that mercury eliminated from other tissues accumulates in the kidneys. Garcia et al. (1974) suggested that this is the case in rat pups prenatally exposed to mercury. In their study, pregnant rats were fed tracer doses of mercury on day 16; pups were sacrificed at birth, 7, 14, 21 and 28 days of age. In the pups, mean mercury concentrations in all tissues except the kidneys were greater than corresponding maternal levels (the mercury levels in maternal and neonatal kidneys were comparable). Of all the pup tissues studied, only the kidneys demonstrated a positive correlation coefficient for concentration against time, suggesting that mercury lost from other fetal tissues is deposited in the developing kidney.

This increased accumulation and retention of mercury in the fetal kidney may render it susceptible to mercury intoxication. Thus the fetal kidney, like its adult counterpart, may be a target organ of mercury. The developing kidney may in fact be more susceptible to injury than the adult kidney (Gibson, 1976). In their study of prenatal mercury nephrotoxicity, Chang and Sprecher (1976) observed more extensive renal lesions in pups born to treated rats than had previously been described in cases of adult mercury nephropathy. They also noted that the degenerative changes in kidneys of neonatal rats prenatally exposed to mercury can still be observed in the adult stage of these animals, suggesting that mercury causes permanent alterations in the development of the kidney.

The morphological and functional development of the mammalian kidney continues well into the postnatal period (Edelmann, 1973; Nash and Edelmann, 1973; Spitzer and Brandis, 1974; Gibson, 1976). Nephrogenesis continues postnatally in most mammals (Spitzer and Brandis, 1974); it has been estimated to continue through the third postnatal week in the dog (Horster et al., 1971) and through the fifth week in the rat (Arataki, 1926). Humans (Nash and Edelmann, 1973; Spitzer and Brandis, 1974) and guinea pigs (Spitzer and Brandis, 1974) are usually born with their full complement of nephrons although those present at birth are, for the most part, incompletely developed, and continue to mature during the postnatal period (Nash and Edelmann, 1973).

Glomerular size increases during the early postnatal period. In human infants, glomerular diameter doubles during renal maturation, with a consequent eight-fold increase in glomerular volume. Tubular development also continues postnatally. In the newborn, loops of Henle are short; as many as 20% are totally within the cortex. During maturation, loops elongate to occupy the primarily medullary location which is typical of the adult kidney (Nash and Edelmann, 1973).

The fetal and neonatal kidney has been shown to have a relatively low functional capacity, including a low rate of glomerular filtration (Gresham et al., 1972; Spitzer and Brandis, 1974; Gibson, 1976) and limited tubular reabsorption and secretion (Edelmann, 1973; Gibson, 1976). Glomerular filtration rate increases with advancing postnatal age (Nash and Edelmann, 1973). In their study of nephron maturation in guinea pigs, Spitzer and Brandis (1974) found the initial increase in

glomerular filtration rate to be a consequence of increased activity of deep nephrons; thereafter, the superficial nephrons appeared to be solely responsible for the increasing rate.

The limited tubular reabsorption and consequent urinary loss of glucose, amino acids, phosphate and bicarbonate observed in immature kidneys (Gibson, 1976) may be due to the limited amount of tubular tissue present (Edelmann, 1973). Edelmann (1973) proposed that there might also be a functional glomerulotubular imbalance, and that reabsorption in the proximal tubule may thus represent a smaller portion of the filtered load than it would later in development, allowing materials primarily or exclusively reabsorbed in the proximal tubule to leak into the urine.

While this immature function is a completely normal part of renal development, immaturity may render the kidney particularly sensitive to toxic injury (Gibson, 1976). Given the ability of mercury to readily cross the placenta and be transferred through the mothers' milk (and thus affect the kidney during all stages of development), the preferential accumulation of mercury in fetal tissues over maternal tissues, and the greater concentration and retention of mercury in the kidney as compared to other tissues, it would appear that the danger to the developing kidney posed by mercury is significant.

CHAPTER 3

MATERIALS AND METHODS

Nulliparous Sprague-Dawley rats, aged 60-90 days, were housed overnight with young adult males of the same strain. Pregnant animals were identified 14 days later by abdominal palpation. Females were then housed in stainless steel metabolism cages until ready to litter, then transferred to plastic littering boxes. Water and Purina Laboratory Chow were provided ad libitum.

Thirty-four rats were treated chronically, beginning on the thirteenth day of gestation (the first day after breeding was considered day zero). Rats were divided into three treatment groups; each received daily subcutaneous injections of (1) mercuric chloride (HgCl_2) at a dose of 4.0 mg/kg body weight, (2) HgCl_2 at a dose of 2.0 mg/kg body weight, or (3) an equal volume of sterile isotonic saline. Injection volume was approximately 0.2 ml, and a new site was used for each injection. To compensate for changes in maternal body weight (recorded every 2 days), either the concentration of the HgCl_2 solution used or the injection volume was adjusted slightly. The concentration of HgCl_2 solutions used ranged from 2.00 to 4.86 mg/ml. Four animals in each treatment group were dosed until littering; the remainder (six rats in the control and low-dose groups, 10 in the high-dose group) were dosed through the first 10 days of lactation.

General health and behavior of the females, as well as weight gain or loss, were monitored during the course of treatment. Urine volume was measured at 24-hour intervals and urine protein and glucose levels

were estimated each day of treatment during gestation using Uristix reagent strips (Ames Laboratories).

At littering, all pups were counted, weighed collectively and examined for external abnormalities. Two pups were randomly selected from each litter for histological evaluation of newborn kidneys. Four of the dams from each treatment group were sacrificed within a day after parturition and their kidneys were prepared for histological evaluation. The remaining pups from these litters were sacrificed and examined for gross internal abnormalities. Their kidneys were removed and weighed collectively.

At 12 days of age, pups were again weighed collectively and examined externally. Two pups from each litter were randomly selected for histological evaluation of their kidneys. The remaining pups were sacrificed, their kidneys were removed and weighed collectively. Dams were also sacrificed at this time and their kidneys prepared for histological examination.

To evaluate the acute nephrotoxic effects of HgCl_2 , adult and fetal kidneys from an additional group of rats were examined after 2 days of treatment. Four adult rats (two at each dose level) were sacrificed after only two subcutaneous injections of HgCl_2 at a dose of either 4.0 or 2.0 mg/kg body weight, and their kidneys were perfused and processed for histological examination. Six pregnant rats (two per treatment group) were injected subcutaneously with HgCl_2 (4.0 or 2.0 mg/kg body weight) or saline on days 17 and 18 of gestation. Females were sacrificed on day 19 and two fetuses from each were randomly selected for histological examination of fetal kidneys.

For a number of reasons, accurate preservation of renal tissue is difficult to achieve. Changes in morphology occur quite rapidly following interruption of the renal blood supply, thus intravascular perfusion fixation was performed to preserve the morphology of the functioning adult kidney (Griffith et al., 1967). Dams were anesthetized with Metofane (methoxyflurane, Pitman-Moore) and kidneys were fixed in vivo by retrograde aortic perfusion. Surgical methods used were similar to those described by Griffith et al. (1967). The perfusion apparatus was set up as shown in Figure 1. One perfusion bottle contained a heparinized isotonic saline wash solution; the other contained 1% glutaraldehyde in Tyrode's solution, the fixative. Fluid reservoir levels were kept at 216 cm above the animal to maintain, via gravity, a constant pressure of approximately 160 mm Hg. The polyethylene tubing was first connected to the wash solution reservoir

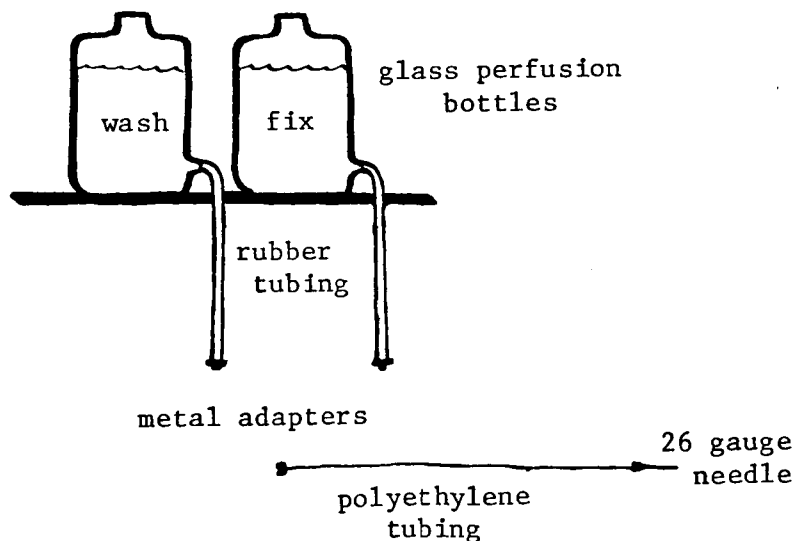


Figure 1. Apparatus used for perfusion fixation of adult rat kidneys.

by means of the metal adapters. The needle was inserted into the abdominal aorta in a retrograde fashion just cephalad to its bifurcation. The aorta was then ligated at the diaphragmatic crus to prevent perfusion of other areas. The renal vein was cut, and the heparinized wash solution was perfused through the vasculature for approximately 30 seconds. With the needle still in place in the aorta, the polyethylene tubing was connected to the fixative reservoir. After perfusion with fixative for 2 to 3 minutes, the kidney was excised. Transverse slices were taken from the mid-hilar region and kept in 10% neutral buffered formalin until processed.

Perfusion fixation proved too difficult to perform on the pups due to their small size. As an alternative, pups were anesthetized with Metofane, and kidneys were fixed in situ by dripping 1% glutaraldehyde in Tyrode's solution on their surface with their blood supply intact. While not as effective as perfusion fixation, this method has been shown to more accurately preserve renal morphology than does fixation after excision of the kidney (Griffith et al., 1967). After fixation, kidneys were excised, sliced in half at the hilus and kept in 10% neutral buffered formalin until processed.

All tissues were dehydrated through a graded series of alcohols, cleared in toluene and paraffin-infiltrated in an automatic tissue processor before being embedded in paraffin. Five-micron sections were cut on a rotary microtome and mounted on glass slides. Sections were stained with either Harris' hematoxylin and eosin, periodic acid-Schiff for the demonstration of renal brush borders, basement membranes and hyaline substances, or Novelli's method (acid fuchsin and light green)

for the demonstration of mitochondria. Staining techniques used were those described by Thompson (1966).

Mercury levels in selected tissues from adult rats that were treated on days 17 and 18 of gestation and their fetuses were estimated by a semi-quantitative method described by Kaye (1975). Pups not used for histological examination were sacrificed and their kidneys and livers were removed. Kidneys from all the pups of a treatment group were pooled, weighed and homogenized. Fetal livers were treated in the same manner. Maternal kidneys were removed, weighed, and homogenized at the time of sacrifice. Acidified tissue homogenates were heated in a flask with a piece of copper, causing mercury in the tissue to be deposited on the copper. The piece of copper was then exposed to cuprous iodide, resulting in the formation of mercury iodide. The color of the mercury iodide varied depending on the amount of mercury present on the copper, and was used to estimate tissue mercury levels by comparison with reference solutions treated in a similar manner.

Standard statistical analyses were performed on the data using single classification analysis of variance and Tukey's procedure for comparison of means (Steel and Torrie, 1960). A 95% level of probability was used in all tests for significance unless otherwise noted.

CHAPTER 4

RESULTS

General behavior of the females varied considerably during the course of the study but no abnormal behavior was observed in any individual. No gross neurological signs of mercury toxicity were observed.

General health of the treated animals was good throughout the study for those in the control and low-dose (2.0 mg/kg) groups, though a few in the latter group suffered mild diarrhea which began on the third day of treatment and lasted 2 to 3 days. Twelve of the 14 high-dose (4.0 mg/kg) rats developed diarrhea within 2 days after treatment began. Four of these exhibited only mild signs for 3 days then recovered completely. The eight others suffered severe diarrhea; of these, three recovered after 3 to 4 days of illness, two had diarrhea throughout treatment, and three died after only 5 or 6 days of treatment. Food and water intake by those that died was greatly reduced in the few days prior to death, as was their urine output.

Several mercury-treated animals developed ulcerous sores at some injection sites. Initially there was local inflammation with skin discoloration and hair loss, followed by the formation of crusty scabs often near 1 cm in diameter. Eventual loss of these scabs revealed the underlying musculature, indicating involvement of both the epidermal and dermal layers. Injections were made at a new site each day, and sores did not develop at every site; many only became mildly inflamed or showed no reaction to the mercury injection. Two dams in the 2.0 mg/kg

group developed ulcers at a few injection sites after several days of treatment. Six high-dose dams were also affected. Their ulcers were more numerous and severe than those of the low-dose animals.

No urinary glucose was detected in any of the control rats throughout treatment. Histograms of urinary glucose levels for each of the mercury-treated rats are shown in Figure 2. A trend can be seen among rats of the high-dose group. In general, there was a marked increase in urine glucose levels on gestational day 14, after only one HgCl_2 injection. Glucose levels remained elevated for 3 or 4 days after treatment began, then decreased, sometimes sharply, to the baseline. In all cases, glucose levels had returned to normal by day 19 in spite of continued daily injections. The low-dose group's histograms differed somewhat from those of the high-dose group. In general, urine glucose levels peaked on either day 14 or 15 of gestation, not consistently as early as high-dose peaks, nor were they consistently as high. Glucose levels remained elevated for a shorter period of time, 1 to 3 days, and returned to normal by day 18.

Urine protein levels in control rats fluctuated between 30 and 100 mg/dl throughout treatment, establishing a rather wide range of normal values. Histograms of urinary protein levels for mercury-treated dams are shown in Figure 3. For most of the 2.0 mg/kg rats, urine protein levels increased above control values on gestational day 14 and remained elevated for 2 to 6 days of treatment. In all cases, protein levels had returned to the normal range by day 20. Urine protein levels of 4.0 mg/kg rats also increased above normal on day 14. In most cases, the increase was comparable to that shown in the low-dose group's

4.0 mg/kg

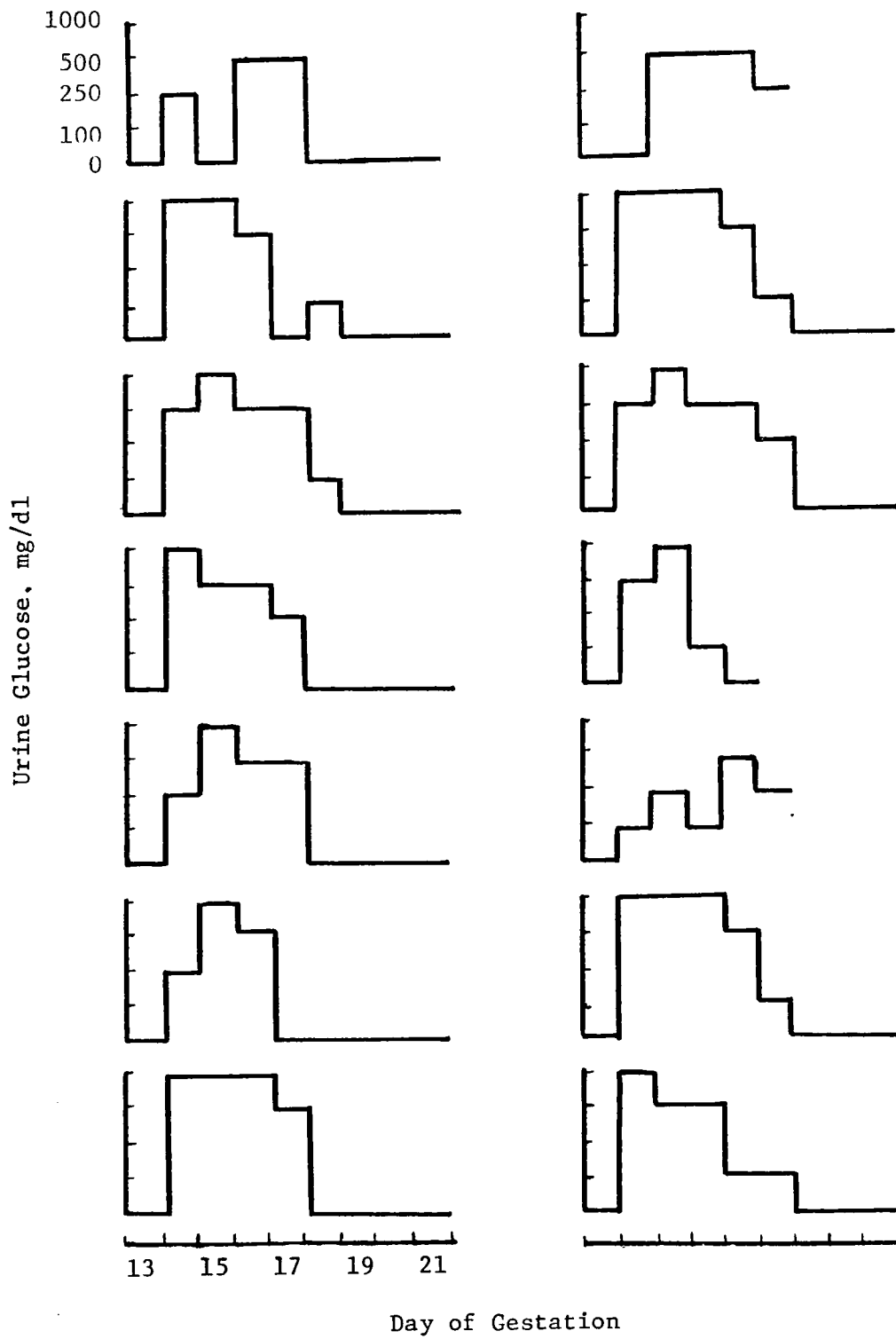


Figure 2. Urinary glucose levels of rats receiving daily injections of HgCl_2 .

2.0 mg/kg

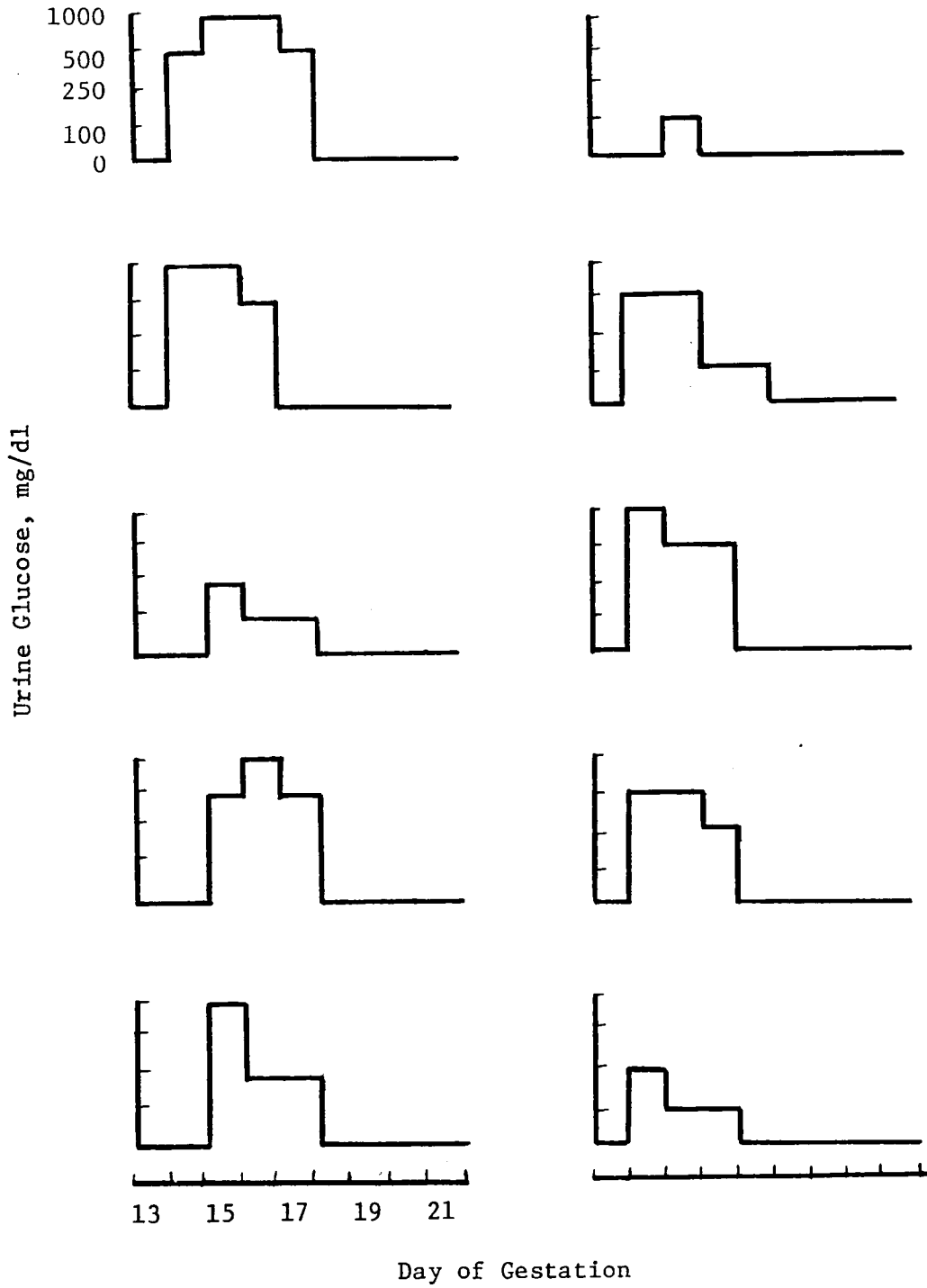


Figure 2 (continued).

2.0 mg/kg

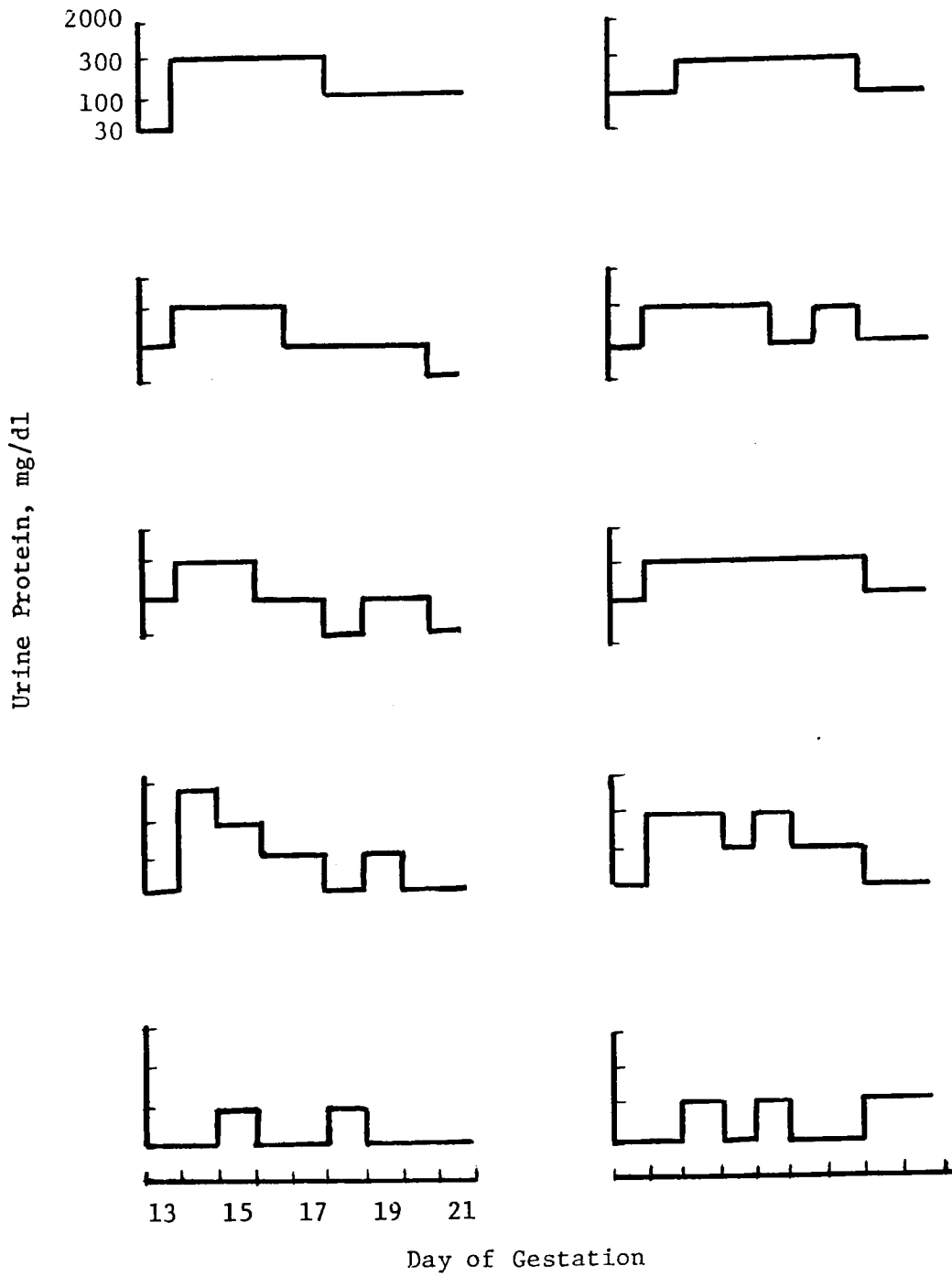


Figure 3. Urinary protein levels of rats receiving daily injections of HgCl_2 .

4.0 mg/kg

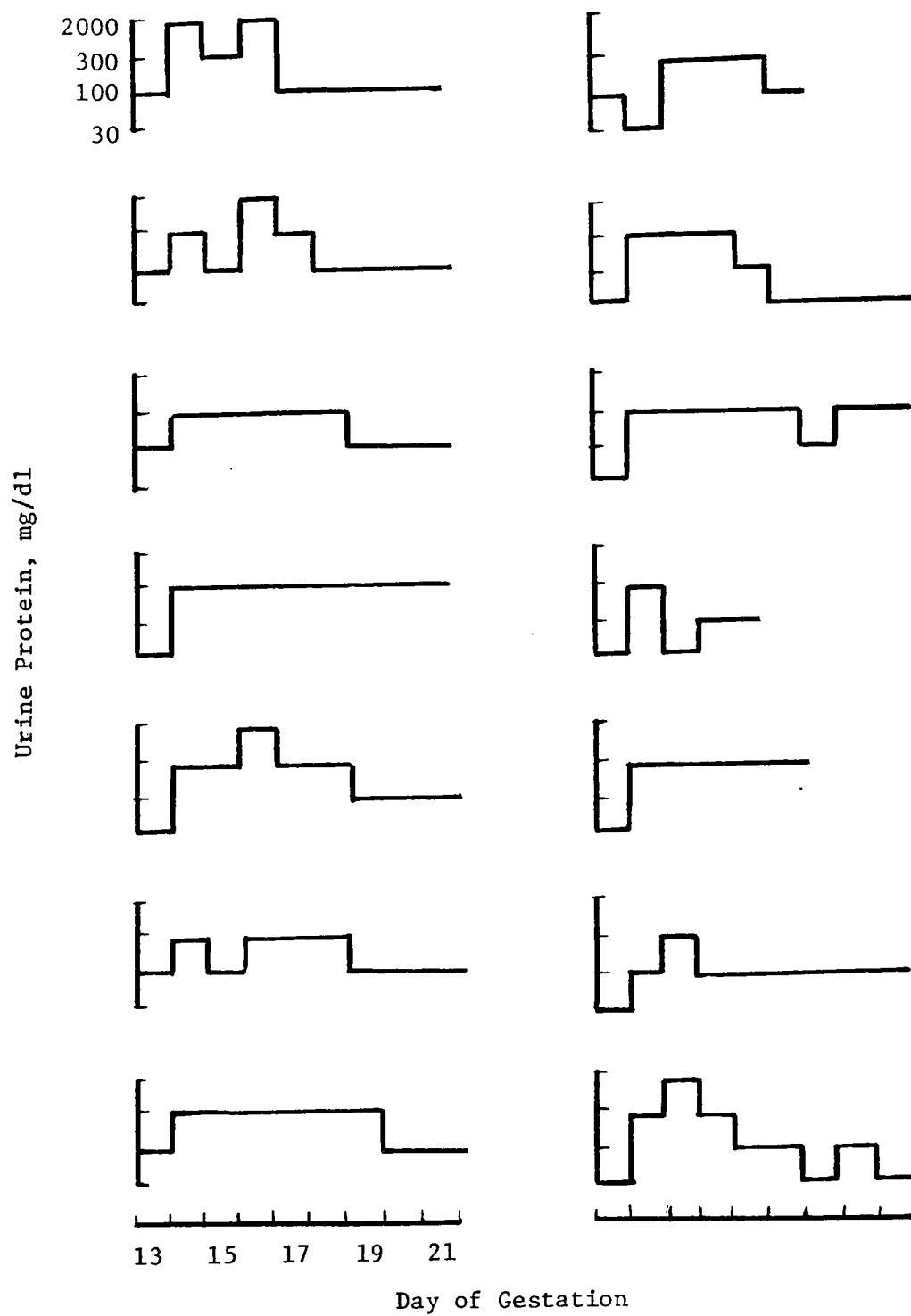


Figure 3 (continued).

histograms, however, protein levels generally remained elevated longer, for 3 to 8 days. In some cases, urine protein levels had not returned to normal by the time of parturition.

Mean urine volumes for each treatment group for the last 7 days of gestation are shown in Figure 4. Both control and low-dose volumes were fairly constant throughout the treatment period and there was no significant difference between the means of these two groups on any day. Mean urine volumes for the 4.0 mg/kg females were comparable to those of the other two groups on the first 2 and last 3 days recorded (days 14 and 15; days 18, 19 and 20) but peaked sharply to significantly higher levels on days 16 and 17 of gestation.

Figure 5 shows the changes in maternal body weight for each treatment group. Both the control and 2.0 mg/kg females gained weight at rates within the normal range for the latter part of pregnancy; however, a modest effect of the low mercury dose is indicated by the consistently lower rate of gain by the rats in this group. Differences between these two groups were not significant at any time. During the first 4 days of treatment, rats receiving 4.0 mg/kg HgCl_2 actually lost weight. Beyond this time they showed a somewhat normal rate of gain but remained significantly below the control level until parturition.

Table 1 presents data for litter size, perinatal deaths and average pup birth weight. Litter size varied among dams but there was no significant difference between treatment groups. Mean values for all groups were within the normal range for this strain of rats in our laboratory colony. The percent perinatal deaths (i.e., pups that were born dead or died within a few hours after birth) did not differ

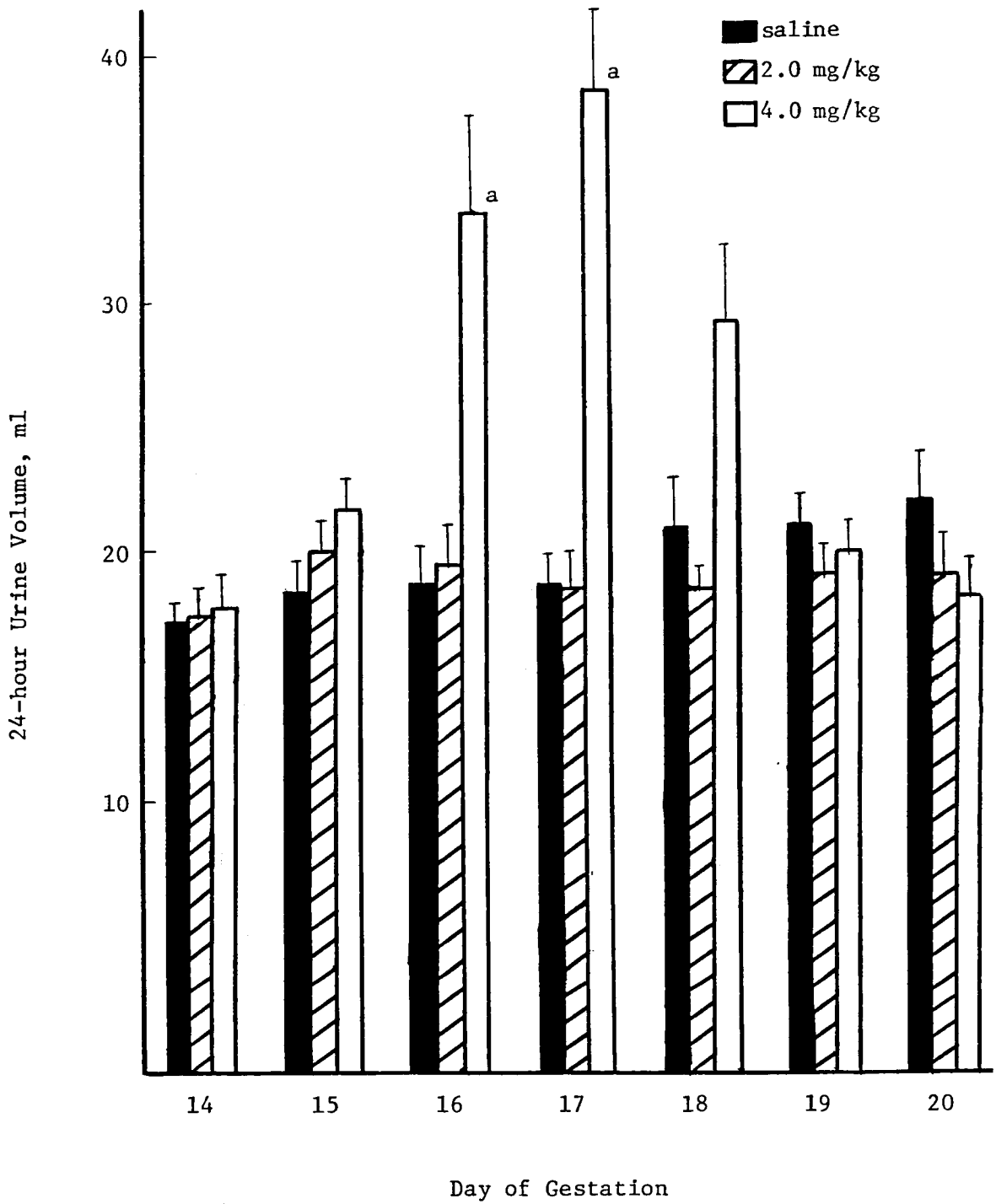


Figure 4. Mean urine volumes ($\pm$ SE) for rats injected daily with HgCl_2 .

^a significantly greater than control mean ($P < 0.05$).

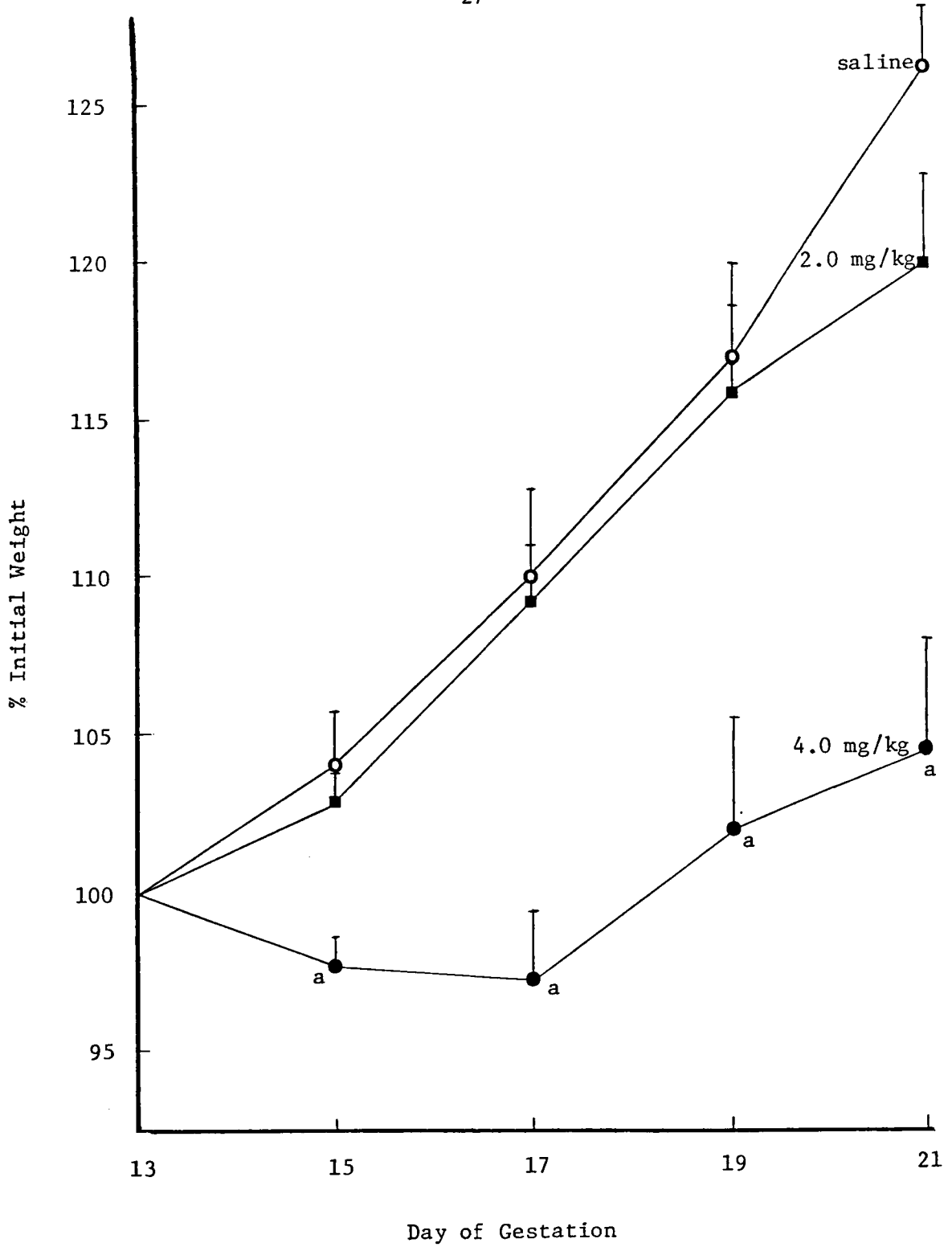


Figure 5. Mean body weights ($\pm$ SE) of rats injected daily with HgCl_2 .

^asignificantly lower than control mean ($P < 0.05$).

Table 1. Litter size, percent perinatal deaths and average pup birth weights for rats treated daily with HgCl₂ beginning on day 13 of gestation.

	4.0 mg/kg/day HgCl ₂				2.0 mg/kg/day HgCl ₂				Saline			
	Litter size	Percent perinatal deaths ^a	Average pup birth weight (g)		Litter size	Percent perinatal deaths	Average pup birth weight (g)		Litter size	Percent perinatal deaths	Average pup birth weight (g)	
Treated through parturition	14	7.1	4.41		12	0.0	5.54		12	0.0	5.54	
	9	0.0	4.57		11	36.4	4.94		11	9.1	6.79	
	13	0.0	4.53		14	7.1	5.44		10	10.0	6.20	
	12 ^b	--	--		13	15.4	4.64		11	18.2	5.44	
Treated through 10 days post-partum	9	0.0	5.68		10	10.0	5.91		12	8.3	5.73	
	14	7.1	4.84		15	6.7	5.53		9	0.0	6.64	
	11 ^c	36.4	4.25		13	15.4	5.00		7	14.3	5.73	
	14	14.3	5.18		11	0.0	6.02		10	30.0	5.87	
	6	100.0	4.22		14	14.3	5.15		11	63.6	5.58	
	10	0.0	4.19		8	12.5	4.74		15	40.0	4.31	
	12	0.0	4.55									
Mean	11.3	16.5	4.64 ^d		12.1	11.8	5.29		10.8	19.4	5.78	
SE	±0.8	±10.0	±0.15		±0.7	±3.3	±0.15		±0.7	±6.3	±0.22	

^ai.e., pups that were born dead or died within a few hours after birth.

^bThese fetuses were removed surgically.

^cThis dam ate all the live pups 1 day after birth; no postnatal data is available for this litter.

^dSignificantly lower than control mean ($P < 0.001$).

significantly among treatment groups. From data on litter size and perinatal deaths, there was no indication of increased fetal mortality due to HgCl_2 treatment. All of the pups examined at birth appeared normal except for one of a low-dose litter that was born without rear legs or a tail. This malformation has been previously reported in mice exposed to mercury in utero (Su and Okita, 1976) and may have been related to HgCl_2 treatment. Since this was the only malformation observed in the study, however, it is difficult to make any inferences.

The HgCl_2 treatment had an effect on pup birth weight. Average birth weight of pups born to low-dose females was somewhat depressed, but not significantly lower than control pup birth weight. A greater effect was seen in high-dose pups, whose mean birth weight was significantly lower than that of the other two groups ($P < 0.001$). Since litter size was comparable in the three treatment groups it can be assumed that the reduced birth weight of high-dose pups was related to the HgCl_2 treatment and not a result of larger litter size. Decreased birth weight as a result of in utero mercury exposure has been previously reported in both rats and mice (Fuyuta et al., 1978; Chen et al., 1979).

Table 2 presents data for the number of pups nursed, pup weight and pup weight gain from dams treated through the first 10 days of lactation. The mean weight of high-dose pups at 12 days of age was significantly lower than that of the other two groups ($P < 0.10$). Since the mean birth weight of high-dose pups was significantly lower than the control mean, the weight gain of pups in each group was calculated as a percent of birth weight. The mean weight gain of high-dose pups was

Table 2. Numbers of pups nursed 12 days, average pup weights at 12 days and average pup weight gains for litters of dams treated with HgCl₂ from gestational day 13 through the first 10 days of lactation.

		4.0 mg/kg/day HgCl ₂			2.0 mg/kg/day HgCl ₂			Saline		
Number of pups nursed 12 days	Average weights (g) at 12 days	Average pup weight gain ^a	Number of pups nursed 12 days	Average pup weights (g) at 12 days	Average pup weight gain	Number of pups nursed 12 days	Average pup weights (g) at 12 days	Average pup weight gain	Number of pups nursed 12 days	Average pup weight gain
7	18.74	229.7	6	22.99	289.5	10	19.94	260.1		
10	15.33	217.0	11	17.98	225.2	7	24.71	272.4		
10	16.70	222.6	9	17.06	241.0	2	24.22	323.0		
8	13.08	212.5	9	20.21	235.9	5	20.92	256.6		
10	17.86	292.9	10	15.82	207.4	1	16.05	187.8		
			11	22.43	372.9	7	17.50	305.7		
Mean	9.0 ^b	16.34 ^b	9.3	19.42	262.0	5.3	20.56	267.6		
SE	±0.6	±1.00	±0.8	±1.20	±24.8	±1.4	±1.42	±19.2		

^aExpressed as a percent of birth weight.

^bSignificantly different from control mean ($P < 0.10$).

slightly lower than the control mean, but differences were not significant. This modest effect on pup growth cannot necessarily be attributed to the HgCl_2 treatment since the 4.0 mg/kg/day dams nursed significantly more pups than did control dams ($P < 0.10$).

Average kidney weights (expressed as a percent of body weight) at birth and at 12 days of age are shown in Table 3. Neither at birth nor at 12 days were kidney weights significantly different among treatment groups.

Kidneys from adult rats appeared grossly normal after 9 or 19 days of treatment. In sections stained with hematoxylin-eosin, renal architecture appeared quite normal in both control and HgCl_2 -treated animals. Under low power, division of the kidney parenchyma into an outer, densely eosinophilic cortex and a much paler-staining inner medulla was clearly defined. The capsule covering the cortex was also visible. The cortex itself was divided into two closely interdigitating zones, the outer, more eosinophilic zone and the inner, somewhat paler staining zone. Under higher magnification, the outer cortex was seen to be composed largely of proximal and distal convoluted tubules and glomeruli, though the immediate subcapsular zone was free of glomeruli. The inner cortex consisted almost entirely of proximal and distal tubules, with glomeruli infrequently seen. Tubular patency varied somewhat among different rats depending on the quality of fixation achieved. Intraluminal nuclei were observed in both control and mercury-treated kidney sections; these were probably agonal artifacts and of no pathological significance (Longley and Burstone, 1963). The medulla contained the terminal portions of tubules, loops of Henle and

Table 3. Average pup kidney weights at birth and at 12 days of age for litters of dams treated 9 or 19 days with HgCl₂.

	4.0 mg/kg/day HgCl ₂		2.0 mg/kg/day HgCl ₂		Saline	
	Average pup kidney weight at birth ^a	Average pup kidney weight at 12 days ^a	Average pup kidney weight at birth	Average pup kidney weight at 12 days	Average pup kidney weight at birth	Average pup kidney weight at 12 days
	0.50 (11) ^b	0.58 (5)	0.41 (10)	0.57 (4)	0.54 (9)	0.70 (8)
	0.47 (7)	0.60 (8)	0.44 (4)	0.60 (9)	0.53 (8)	0.57 (5)
	0.52 (11)	0.59 (8)	0.41 (11)	0.62 (7)	0.52 (7)	0.60 (1)
		0.58 (6)	0.52 (9)	0.62 (7)	0.48 (7)	0.60 (3)
		0.61 (8)		0.72 (8)		0.62 (5)
				0.65 (2)		
Mean	0.50	0.59	0.45	0.63	0.52	0.62
SE	±0.01	±0.01	±0.03	±0.02	±0.01	±0.02

^aKidney weights are expressed as a percent of pup body weight; values given represent an average of both kidneys from all live pups of each litter, except those used for histological evaluation.

^bNumbers in parentheses indicate the number of pups used in calculating the average kidney weight.

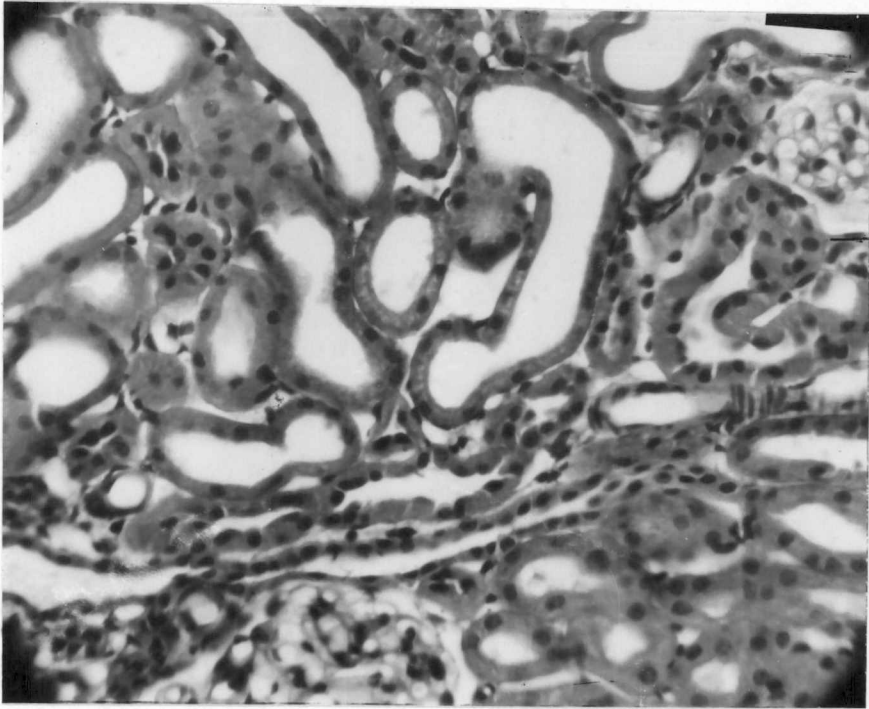
collecting tubules. Interstitial tissue was more abundant in the medulla than in the cortex.

Small areas of interstitial fibrosis were found in kidneys from rats receiving 9 or 19 HgCl₂ injections. Early regenerating tubules were also present; these were lined by densely packed, low cuboidal epithelial cells and were often surrounded by numerous inflammatory cells. Mitotic figures were occasionally found in these regenerating tubular cells. Examples of regenerating tubules are shown in Figure 6.

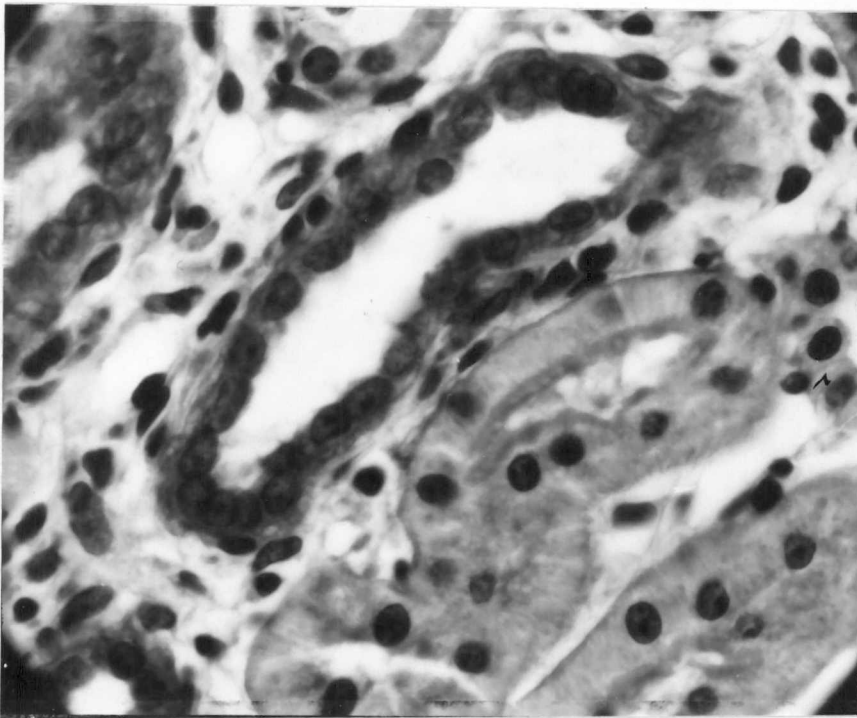
Kidney sections stained with periodic acid-Schiff (PAS) revealed intact tubular brush borders and basement membranes. The demonstration of brush borders enabled a clear distinction to be made between the proximal and the distal tubules of the outer cortex. With the PAS method, a distinction between outer and inner cortical zones was also apparent; inner cortical proximal tubules showed more strongly PAS-positive brush borders than did proximal tubules of the outer zone. No hyaline casts or droplets were observed in PAS-stained kidney sections from control rats; hyaline droplets were occasionally seen in those treated with HgCl₂ for 9 or 19 days as was thickening of the tubular basement membrane.

In kidney sections stained by Novelli's method for mitochondria a distinction between outer and inner cortex was apparent; mitochondria were more numerous in outer cortical tubules, thus these tubules stained more deeply purple than the tubules of the inner cortex. The medulla stained green and light purple, indicative of the sparsely packed mitochondria. In kidney sections from rats treated with 4.0 mg/kg HgCl₂ for 9 days, large areas of tubules that did not stain at all for

Figure 6. Photomicrographs of a kidney section from a rat treated with 4.0 mg/kg HgCl_2 for 9 days. A. Early regenerating tubules lined by low cuboidal epithelial cells. Normal tubules, with a typical columnar epithelium are on the right (PAS stain, 380X). B. Higher magnification (620X) of an area of the same section. Note the crowded nuclei of the regenerating epithelium and the thickened tubular basement membrane.



A



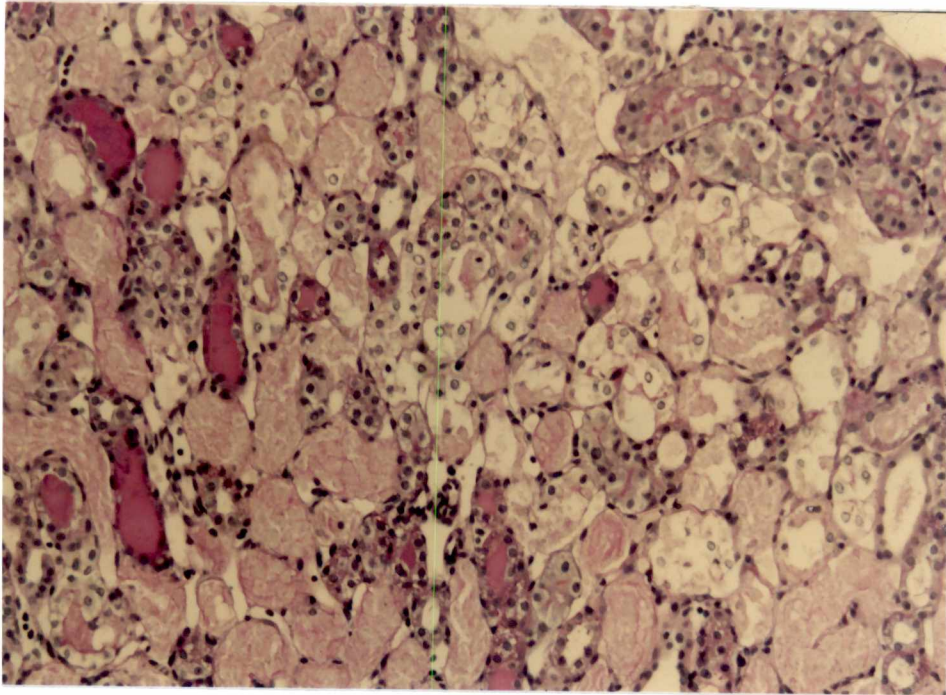
B

Figure 6

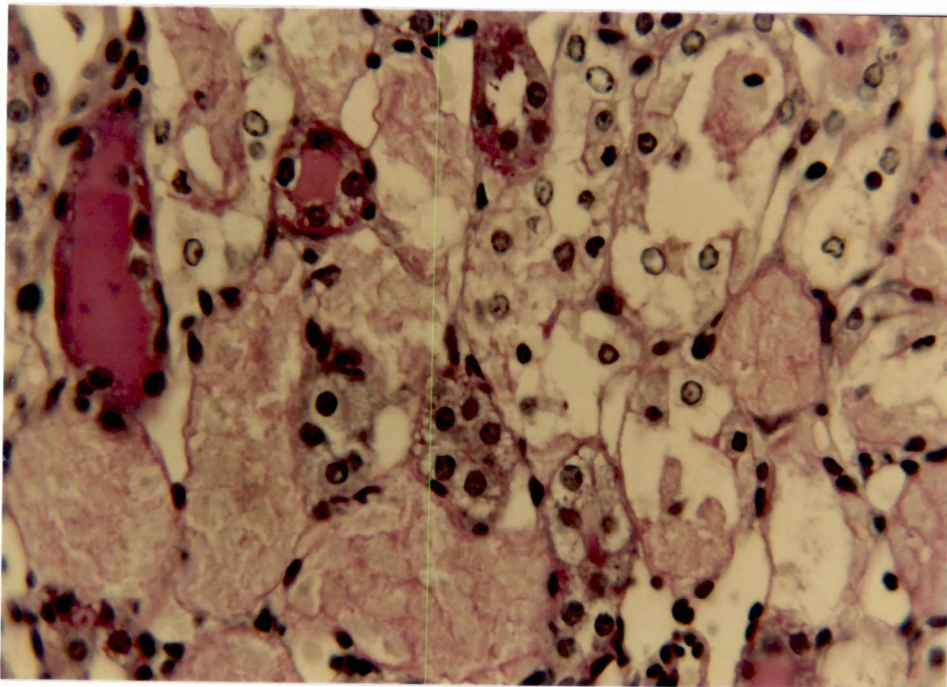
mitochondria were observed. These green-staining tubules were found in the inner cortex adjacent to normal, deeply staining purple tubules of the outer cortex. The early regenerating tubules, characterized by a flattened epithelium and numerous nuclei, also stained green in contrast to the morphologically normal tubular cells which stained purple.

Kidneys from adult rats sacrificed after two daily injections of HgCl_2 revealed marked histological differences from control kidneys. In rats receiving 4.0 mg/kg HgCl_2 , widespread areas of necrotic tubular cells were found in the inner cortex of kidney sections stained with hematoxylin-eosin. There was a complete necrosis in these areas, with darkening of the cytoplasm and absence of nuclei in the epithelial cells. The outer cortex was apparently unaffected. This localization of damage in the inner cortex reflects primarily pars recta injury and is consistent with the observations of many other investigators. Areas of apparently normal cells surrounded the necrotic patches. Staining with PAS revealed occasional disruption of the brush border and numerous hyaline droplets in the cytoplasm of otherwise normal cells located adjacent to the necrotic zone. Hyaline casts in tubular lumens were observed most frequently in the inner cortex and occasionally in the outer cortex and medulla. Tubular basement membranes were intact and there was no apparent glomerular involvement. Necrotic areas are shown in Figure 7. Morphology appeared much more normal in kidney sections from low-dose rats. Small, scattered areas of necrosis were observed in the inner cortex of sections stained with hematoxylin-eosin. Otherwise normal tubular cells were found to contain numerous hyaline droplets

Figure 7. Photomicrographs of a kidney section from a rat treated with 4.0 mg/kg HgCl_2 for two days. A. Necrotic tubules stain deeply eosinophilic and lack nuclei. Normal tubules can be seen in the upper right, and hyaline tubular casts to the left (PAS stain, 210X). B. Higher magnification (350X) of an area of the same section. Note the persistence of the basement membranes and occasional intact epithelial cells in the necrotic tubules. Hyaline tubular casts are to the left.



A



B

Figure 7

when stained with PAS. Hyaline casts were infrequently seen in the inner cortex and the brush borders and basement membranes remained intact.

Histological examination of kidneys from newborn rats exposed to HgCl_2 in utero revealed no identifiable differences from control pup kidneys by light microscopy. Kidney sections from each of the treatment groups demonstrated the centrifugal pattern of maturation that is typical of the mammalian kidney. In hematoxylin-eosin stained sections, glomeruli at various stages of development could be seen, with the most primitive form (the pronephron) found in the immediate subcapsular zone and the more mature forms located deeper in the cortex. The most fully developed glomeruli, those of adult form, were in the juxtamedullary region. The subcapsular zone, which contained pronephrons surrounded by nephronogenic cells, stained deeply purple. The outer cortex was also basophilic and contained very few tubules; these were small with crowded nuclei and very little cytoplasm in the epithelial cells. Deeper in the cortex, clusters of eosinophilic tubules were observed surrounding glomeruli of more mature forms. When stained with PAS, these tubules demonstrated brush borders, but tubules in the outer cortex were not PAS-positive. Staining with Novelli's method demonstrated the presence of mitochondria in tubular cells of the inner cortex; only a few scattered epithelial cells of the outer cortical tubules stained for mitochondria. Interstitial tissue was observed at the cortico-medullary junction and the medulla was easily distinguishable from the cortex due to its paler staining characteristics.

Kidneys from 12-day-old pups differed markedly from newborn kidneys, demonstrating the rapid growth and maturation of this organ; however, kidney sections from 12-day-old pups in each treatment group appeared essentially the same and no treatment differences were identified by histological examination. In 12-day-old rat kidney sections stained with hematoxylin-eosin, no pronephrons were found and the glomeruli present were in general more mature, with a greater proportion in adult form, than was observed in the newborn kidney sections. By 12 days of age, the subcapsular nephronogenic zone was gone and the immediate subcapsular space lacked glomeruli as is typical of the adult kidney. Distinction between the outer and inner cortex could be made, but was not as well-defined as in the adult kidney sections. Tubules in the outer cortex were larger than at birth, and epithelial cells contained more cytoplasm and thus stained more eosinophilically than did those in newborn kidney sections. Some of these outer cortical tubules demonstrated brush borders when stained with PAS. Novelli's stain demonstrated greater numbers of mitochondria in these tubular cells than were present at birth.

Nineteen-day fetal kidneys were obviously less mature than newborn kidneys, as indicated by the relatively fewer nephrons present and the abundance of interstitial tissue in sections of fetal kidneys. Control and HgCl₂-exposed kidney sections appeared essentially the same when stained with hematoxylin-eosin or PAS. Tubular cells did, however, appear somewhat larger, and basement membranes were more PAS-positive in control sections than in treated sections. One difference between treatment groups was observed in kidney sections stained by Novelli's

method for mitochondria. Sections from HgCl_2 -exposed fetuses did not stain at all for mitochondria, and the entire section was green as a result of the counterstain; control sections stained deeply purple, and the color difference was discernible with the unaided eye.

Interpretation of this difference was difficult since structures other than mitochondria, such as glomeruli and the cytoplasm of tubular cells, stained purple in control sections whereas they normally would not be expected to.

Nineteen-day fetuses, whose dams had received HgCl_2 injections on days 17 and 18 only (at 4.0 or 2.0 mg/kg), were shown to contain measurable levels of mercury in their kidneys and livers. The mercury level in kidney homogenate from fetuses in the 4.0 mg/kg group was estimated at 140 to 185 $\mu\text{g/g}$ tissue; the level in low-dose fetal kidneys was somewhat lower at 100 to 150 $\mu\text{g/g}$ tissue. Liver homogenates from each group had lower levels of mercury than corresponding kidney levels, approximately 8 $\mu\text{g/g}$ tissue in the 4.0 mg/kg group and <8 $\mu\text{g/g}$ tissue in the 2.0 mg/kg group. On a per weight basis, the fetal kidneys contained much higher levels of mercury than the fetal livers. Maternal kidneys were estimated to contain approximately 12 to 18 $\mu\text{g/g}$ tissue after two injections of HgCl_2 at 2.0 mg/kg and 17 to 22 $\mu\text{g/g}$ tissue after two doses at 4.0 mg/kg.

CHAPTER 5

DISCUSSION

Daily subcutaneous injections of HgCl_2 at 2.0 or 4.0 mg/kg produced identifiable toxic effects in pregnant rats. While no neurological signs of mercury intoxication were observed in any rats, many of the 4.0 mg/kg group were at least moderately ill, and several died, presumably as a result of the mercury treatment. The diarrhea commonly exhibited by treated rats was evidently due to the effects of mercury on the gastrointestinal tract. Hunter (1929) has reported a marked diphtheritic enterocolitis so severe in rabbits as a result of HgCl_2 treatment (by subcutaneous injection) that the animal seemed to suffer as much from the intestinal lesions as from nephritis. An enterocolitis could account for the diarrhea, weight loss and depressed rate of weight gain observed in many of the HgCl_2 -treated females. While the brain and kidney have long been considered target organs of mercury (Ware et al., 1974a,b; Chang, 1977; Hammond and Beliles, 1980), it is evident from these results that the gastrointestinal tract is also adversely affected by HgCl_2 . Furthermore, these results indicate that the metal is excreted through the colon as well as through the kidney, as has been suggested previously (Berlin and Ullberg, 1963; Hammond and Beliles, 1980).

The ulcers that developed at injection sites on some of the HgCl_2 -treated rats are consistent with an early report of local inflammation and necrosis of tissue at the site of subcutaneous HgCl_2 injections (Hunter, 1929). This local reaction probably indicates that

some of the HgCl_2 is retained locally and thus does not reach the kidney. With evidence of some intestinal elimination of mercury, it is apparent that only a portion of the dose reached the target organ in this study.

No nephrotoxic injury was identified in histological specimens from adult rats treated with HgCl_2 for 9 or 19 days (at 2.0 or 4.0 mg/kg), though the urinalysis results from these animals indicated some degree of renal functional impairment during the course of treatment. Proteinuria and glucosuria are two of the most useful indices of nephrotoxicity, especially when occurring together. Proteinuria can accompany both glomerular damage and proximal tubular necrosis; that associated with tubular injury is characteristic of heavy metal nephrotoxicity (Piperno, 1981). Glucosuria is also known to accompany proximal tubular damage induced by heavy metals (Hook, 1980). The mechanism of urinary protein and glucose loss in response to tubular injury is not well understood. Hook (1980) has suggested that impaired tubular function may account for the glucosuria frequently observed in cases of tubular necrosis. This may not adequately explain the glucosuria accompanying mercury intoxication since mercury has been shown to produce relatively selective damage to the pars recta of the proximal tubule (Rodin and Crowson, 1962a; Verity and Brown, 1970; Fowler, 1972; Ganote et al., 1975; Ware et al., 1975) where only a small amount of glucose reabsorption occurs (most glucose is reabsorbed in the pars convoluta). Thus, damage to the pars recta would not be expected to impair glucose reabsorption to a very great degree. The marked glucosuria observed to accompany pars recta injury in this study and in

earlier research (Carone and Ganote, 1975) may be due not to impaired tubular reabsorption of glucose, but to its diffusion from the interstitial to the tubular fluid across the denuded permeable basement membranes of necrotic tubules. At higher doses of mercury, the entire proximal nephron is affected, and under these circumstances impaired reabsorption may contribute to urinary glucose loss.

Micropuncture studies indicate that both glucosuria and proteinuria associated with tubular necrosis are due to the backward diffusion of these materials into the tubular fluid across abnormally permeable basement membranes of necrotic tubules (Carone and Ganote, 1975). Abnormal permeability to Lissamine green (a dye) and inulin has also been demonstrated in the proximal tubules after HgCl_2 treatment (Bank et al., 1967).

The transient polyuria observed in the 4.0 mg/kg dams as well as the marked glucosuria and proteinuria coincided with the extensive tubular necrosis seen in rat kidneys examined after only two HgCl_2 injections. Later in the course of chronic treatment, urine measurements returned to normal and kidneys examined at later times (after 9 or 19 days of treatment) appeared normal, having been initially damaged but then repaired.

Repair and regeneration of the kidney after the acute, necrotic phase of mercury intoxication has been demonstrated previously (Oliver, 1953; Cuppage and Tate, 1967; Biber et al., 1968; Kramp et al., 1974), and several investigators have characterized the reparative phase (Oliver, 1953; Cuppage and Tate, 1967). Parameters often used as indices of tubular necrosis (e.g., cell exfoliation, proteinuria,

glucosuria) can also be useful indicators of tubular regeneration. The pattern of cell exfoliation following nephrotoxic insult has been shown to correspond closely to the pattern of histological necrosis and repair. The peak renal tubular cell response coincides with the onset of massive tubular necrosis and the subsequent fall in cell counts is associated with diminishing tubular necrosis and increasing tubular repair (Prescott and Ansari, 1969). Proteinuria and glucosuria have been shown to develop at the onset of tubular necrosis and then disappear when the tubules are completely relined with new epithelium (Carone and Ganote, 1975). The transient glucosuria and proteinuria reported here are consistent with a histological pattern of initial tubular damage followed by a period of regeneration.

Evidence of tubular regeneration was found in kidney sections from some of the females receiving 4.0 mg/kg HgCl_2 for 9 or 19 days. Small areas of tubules lined by densely packed, flattened cuboidal epithelial cells were found amidst morphologically normal tubules. The early regenerating tubules did not stain by Novelli's method for mitochondria, indicating that they were not functionally mature. Use of Novelli's stain for mitochondria revealed patches of tubular cells in the cortex of some sections that contained few or no mitochondria. These tubular cells had apparently regenerated to the point of normal morphological appearance by routine staining methods but were not yet fully matured. Tubular cells require a period of maturation after their development in which to acquire the organelles and enzymes necessary for normal function (Oliver, 1953; Cuppage and Tate, 1967). It is noteworthy that the kidneys in the present study were capable of regeneration despite

the continued administration of HgCl_2 . Chronic injury was indicated by the small areas of interstitial fibrosis and the numerous inflammatory cells observed in kidney sections from rats treated with HgCl_2 for 9 or 19 days. Similar regeneration during chronic HgCl_2 treatment has been reported, and the development of renal tolerance to mercury has been suggested as a possible explanation for this regeneration (Prescott and Ansari, 1969). Similar tolerance to the nephrotoxic effects of salicylates, bacitracin and cephaloridine has been described (Miller et al., 1960; Scott et al., 1963; Atkinson et al., 1966), and Hunter (1929) demonstrated a slight degree of acquired immunity to mercury in regenerated renal tubular epithelium of rabbits chronically treated with HgCl_2 .

The modest effects of HgCl_2 treatment on the offspring of pregnant rats in this study were not particularly surprising. The embryocidal and teratogenic effects of mercury have been reported by numerous investigators (Khera and Tabacova, 1973; Gilani, 1975; Su and Okita, 1976; Fuyuta et al., 1978; Chen et al., 1979), however, several important differences exist between these studies and the present research which may account for the lack of embryocidal or teratogenic effects reported here. Much of the previous research has used organic forms of mercury, namely methylmercury, which has been shown to cross the placenta more readily than inorganic mercurials (Suzuki et al., 1967), and in general, a larger, single dose of the metal was administered to the female much earlier in gestation. The most important factor may be the time of gestation since the more mature fetus has been shown to be somewhat better protected from non-essential

elements, including mercury, than is the early fetus (Schulert et al., 1969). The treatment period was chosen to coincide with the period of developmental maturation of the fetal kidney, after renal organogenesis was complete. In this way, it was thought that mercury exposure would not result in gross anatomical defects of the kidneys (or other organs), but would perhaps interfere with the functional and morphological maturation.

The histological effects of HgCl_2 -exposure on the developing kidney were difficult to evaluate due to the limited quality of fixation that could be achieved with fetal, newborn and 12-day-old animals. No nephrotoxic effects were identified in newborn rats after exposure to HgCl_2 for 9 days in utero or in 12-day-old pups exposed in utero for 9 days and through lactation for an additional 10 days. The transplacental and lactational transfer of mercury to developing offspring has been well-documented (Berlin and Ullberg, 1963; Suzuki et al., 1967; Suzuki et al., 1971; Yang et al., 1972b; Garcia et al., 1974; King et al., 1976), and the accumulation of mercury in the fetal kidney as a result of in utero exposure has been demonstrated (Garcia et al., 1974). In the present study, the presence of mercury was demonstrated in fetal kidneys after two days of in utero exposure. Given this, and the nephrotoxicity of HgCl_2 that is well-known in adult animals (Rodin and Crowson, 1962a,b; Gritzka and Trump, 1968; Ware et al., 1973; Fowler, 1974; Ganote et al., 1975; Ware et al., 1975; Hook, 1980), it was reasonable to expect some renal damage or maldevelopment in the rat pups of this study. The apparent lack of such effects observed in newborn and 12-day-old kidneys could be explained in several ways.

First, though the presence of mercury in fetal kidneys was confirmed, it is possible that tissue levels were insufficient to produce nephrotoxic effects. Also, the possibility exists that HgCl_2 produced effects in the perinatal kidneys that were too subtle to be detected by light microscopy. The only report available in the literature of renal damage in rat pups exposed to mercury in utero (Chang and Sprecher, 1976) was based on ultrastructural evidence observed by electron microscopy. A valid comparison of the present study with that of Chang and Sprecher (1976) is difficult since they administered a single dose of methylmercury to the dam on the eighth day of gestation and thus were treating an organ at a much different stage of development. Finally, the developing kidney may respond to HgCl_2 in a manner similar to that demonstrated in maternal kidneys in this study, that is, fetal kidneys may have initially been damaged by HgCl_2 but, by the time of histological evaluation have been repaired. An attempt to test this possibility was made by injecting pregnant rats with HgCl_2 for 2 days and examining fetal kidneys immediately thereafter. The only identifiable difference among treatment groups was the reaction to a staining method specific for the demonstration of mitochondria, however, these results were inconclusive due to the rather indiscriminate staining reaction shown in control kidney sections.

In recent research, Kavlock and Gray (1983) have demonstrated that a single subcutaneous injection of HgCl_2 at levels shown to be nephrotoxic to adults, when administered to day-old rat pups failed to alter renal function or morphology when evaluated 2 or 5 days later. These results were similar to those reported in neonatal rats exposed to

an organic mercurial (Wachstein and Robinson, 1965). It is possible that in the present study and in previous ones, the apparent lack of toxicity observed is due to the centrifugal nature of renal development in which successive development of the more superficial nephrons would mask injury to the deeper, earlier-formed, nephrons (Cowan et al., 1980). Thus the normal developmental process of the kidney could serve to protect it from nephrotoxicity (Kavlock and Gray, 1983), and such a series of events could be responsible for the lack of toxicity observed in fetal and neonatal rats of the present study.

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