This is to certify that the

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The Effects of Cisplatin on Carbohydrate Metabolism

presented by

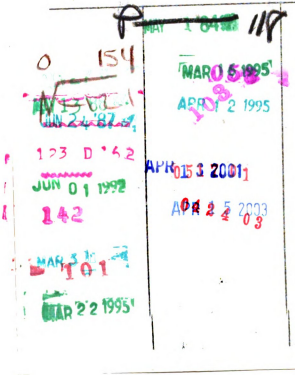
ROBIN SHERYL GOLDSTEIN

has been accepted towards fulfillment
of the requirements for

Ph.D. degree in Nutrition

Jenny T. Bond
Major professor

Date February 12, 1982.



THE EFFECTS OF CISPLATIN ON CARBOHYDRATE METABOLISM

by

Robin Sheryl Goldstein

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
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ABSTRACT

THE EFFECTS OF CISPLATIN ON CARBOHYDRATE METABOLISM

by

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This study was designed to characterize the effects of cisplatin (cis-DDP) and other divalent platinum compounds on carbohydrate metabolism and to elucidate the underlying biochemical and endocrine mechanisms.

Glucose metabolism was evaluated in male F-344 rats treated with equimolar platinum doses of cis-DDP, trans-DDP or ammonium tetrachloroplatinate. A group of pair-fed controls was also studied to correct for the metabolic effects of reduced food intake. Administration of cis-DDP, but not trans-DDP or tetrachloroplatinate, resulted in fasting and nonfasting hyperglycemia. Impaired glucose utilization contributes in part to cis-DDP hyperglycemia, as indicated by a marked and persistent hyperglycemia following an exogenous glucose load in treated animals. Glucose intolerance was apparent 2 and 4, but not 7 and 14, days following cis-DDP (5 mg/kg) treatment, indicating that it is a transient phenomenon. cis-DDP glucose intolerance was accompanied by a relatively deficient plasma insulin response. Neither trans-DDP nor tetrachloroplatinate impaired glucose tolerance at any time examined, suggesting that cis-DDP glucose intolerance is unique to the geometry

of the complex and is related to properties other than the presence of a divalent platinum atom.

Mannitol pretreatment reduced cis-DDP nephrotoxicity and glucose intolerance, suggesting that impaired renal function by cis-DDP contributes in part to the observed glucose intolerance. Administration of other nephrotoxicants, however, did not uniformly impair glucose intolerance.

cis-DDP treated animals did not exhibit a blunted hypoglycemic response to exogenous insulin. Rather, decreased glucose utilization following cis-DDP treatment was associated with impaired insulin secretion, an effect not related to starvation, adrenal-mediated stress, hypokalemia or hypocalcemia. No histopathological damage of cis-DDP treated pancreata was evident.

cis-DDP treatment resulted in hyperglucagonemia, an effect not associated with increased hepatic or renal gluconeogenic enzyme activities. A component in addition to the 3500 MW form of plasma immunoreactive glucagon was significantly elevated by cis-DDP treatment. cis-DDP hyperglucagonemia was reduced by ameliorating cis-DDP nephrotoxicity via mannitol pretreatment. Other nephrotoxicants also increased plasma glucagon. These results suggest that cis-DDP hyperglucagonemia is probably related to decreased renal degradation of glucagon.

In summary, the results of this study indicate that cis-DDP induces hyperglycemia in association with impaired glucose utilization; the latter is mediated by decreased insulin secretion.

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To my mother

for teaching me the meaning of perseverance

To my father

for teaching me the values of an education

To my brother and sister

for their unconditional love and support

and to my husband

for making it all worthwhile

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INTRODUCTION

Cisplatin (cis-dichlorodiammineplatinum) is the first of a group of platinum coordination complexes to be introduced as a cancer chemotherapeutic agent. This agent, both alone or in combination chemotherapy, has been demonstrated to be effective in the treatment of testicular, ovarian, bladder, prostate, lung, and head and neck cancers. However, clinical use of cisplatin is often limited by its toxic side effects, including renal toxicity, gastrointestinal disturbances, myelosuppression and ototoxicity (Madias and Harrington, 1978; Von Hoff et al., 1979).

In addition, administration of cisplatin has also been reported to induce hyperglycemia in laboratory rats (Kociba and Sleight, 1971). Several divalent metal ions are known to induce hyperglycemia by affecting pancreatic islet function (Ghafghazi and Mennear, 1975; Horak and Sunderman, 1975a,b); therefore, a divalent platinum compound such as cis-DDP may similarly impair carbohydrate metabolism. Although an extensive number of studies have characterized specific organ toxicities of cisplatin, very little is known about its effects on intermediary metabolism. The primary objective of the research reported herein is to elucidate the effects of cisplatin and other divalent platinum compounds on carbohydrate metabolism. Implicit in this objective

is an assessment of the biochemical and endocrine correlates of platinum toxicity.

CISPLATIN

History and Development

The biological effects of platinum complexes were first noted in Escherichia coli (E. coli) exposed to an electrical field generated across two platinum electrodes (Rosenberg et al., 1965). Voltage applied across these electrodes resulted in a changed appearance of E. coli from its normal rod shape to an elongated filament form (Rosenberg et al., 1965). Further investigations revealed that one or more long-lived chemical species were produced by the electrical current and induced bacterial filamentation by inhibiting cellular division but not cellular growth (Rosenberg et al., 1965). These chemical by-products were later identified as cis-dichlorodiammineplatinum (II) (cis-DDP) and cis-tetrachlorodiammineplatinum (IV). Further studies established that neutral platinum complexes, such as cis-DDP, not only specifically inhibit cell division without affecting growth rate but derepress latent viral genomic information in lysogenic bacteria (Reslova, 1972), an effect commonly observed with other anti-tumor agents.

On this basis, Rosenberg postulated that neutral platinum complexes, by virtue of their action on bacteria cells, may similarly inhibit cell division in rapidly growing cancer cells. This postulate was confirmed by the successful tumor regression of solid sarcoma 180 in mice by cis-DDP treatment (Rosenberg et al., 1969). Since then, the broad spectrum anti-tumor activity of cis-DDP has been well

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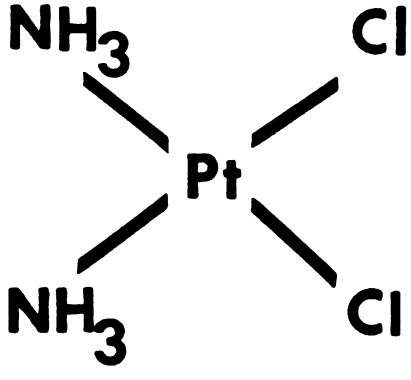
established as indicated by its effectiveness against transplantable, chemical and viral induced tumors, slow as well as rapidly growing tumors, and disseminated as well as solid tumors (Rosenberg, 1978a). Currently, cis-DDP, either alone or in combination chemotherapy, is primarily used in the management of testicular and ovarian cancers, although its use has also been extended to treating cancers of the head and neck, bladder, prostate and lung.

Chemistry and Mode of Action

cis-DDP is a square planar coordination complex containing a central platinum atom surrounded by two chloride atoms and two ammonia moieties (MW = 300.1) (Figure 1). It has a melting point of approximately 270°C and is soluble in aqueous vehicles at maximum concentrations of 1 mg/ml. cis-DDP appears to be highly reactive in in vitro systems. Several reports clearly demonstrate that aqueous solutions of cis-DDP degrade via nucleophilic displacement of the chloride ligands by water molecules (Greene et al., 1979; Lee and Martin, 1976), yielding various combinations of aquated and/or hydroxylated species of the parent drug (Figure 2). The predominating species appear to be determined by pH; the dihydroxy species are formed at a pH greater than 7.37 while the diaquo species are formed at a pH less than 5.51.

In vitro, the lability of the chloride ligands appears to be related to the chloride concentration of the solvent such that addition of chloride to aqueous solutions of cis-DDP results in an increased proportion of intact cis-DDP molecules (Greene et al., 1979). Conversely, preparation of cis-DDP solutions in water with no added chloride



cis-DDP

Figure 1. cis-Dichlorodiammineplatinum (II).

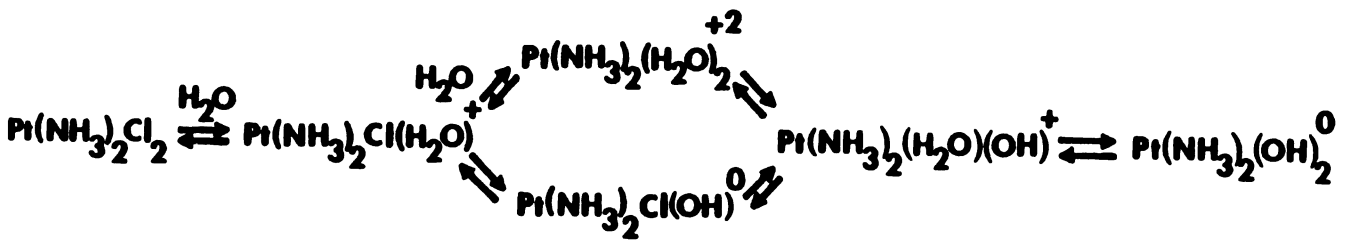


Figure 2. Aquation of cis-DDP.

increases the aquated/hydroxylated complexes (Greene et al., 1979). Furthermore, the reversibility of this reaction has been indicated by the reformation and stabilization of cis-DDP following addition of 0.9% NaCl to an already degraded sample of cis-DDP (Greene et al., 1979). Extrapolating these in vitro data to the in vivo disposition of cis-DDP has led to the hypothesis that cis-DDP exists as an uncharged neutral complex in extracellular fluid (ECF) since the chloride concentration of ECF (≈112 mM) is sufficiently high to stabilize the complex and prevent hydrolysis. However, the markedly lower intracellular concentration of chloride (≈4 mM) facilitates the displacement of chloride by water molecules, yielding a positively charged aquated/hydroxylated platinum complex (Rosenberg, 1978). It is the aquated/hydroxylated intracellular complex which is believed to be active in inhibiting DNA synthesis.

The screening of cis-DDP and other platinum analogues for anti-tumor activity has provided valuable information regarding structure-activity relationships. One striking feature of these studies is that the geometry of these complexes is clearly a determinant of anti-tumor activity, as indicated by the marked anti-tumor activity of the cis, but not the trans, configuration of DDP (Leonard et al., 1971). Furthermore, it has been suggested by Cleare and Hoeschele (1973) that complexes exhibiting anti-tumor activity share definite chemical properties: (1) they are electrically neutral (although the ultimate active form may be charged following ligand exchange in vivo), (2) they exchange only some of their ligands rapidly, (3) the complexes are

either square planar or octahedral, (4) they contain a pair of cis-monodentate (or one bidentate) leaving groups (corresponding trans isomers are generally inactive), (5) the leaving groups are spaced approximately 3.3 Å apart, and (6) ligands trans to the leaving groups appear to be strongly bound and inert. The lability of the cis leaving groups is believed to be important in determining anti-tumor activity and is consistent with the pbstulate that the anti-tumor effects of cis-DDP are mediated by a modified form of cis-DDP. The aquated/hydroxylated forms of cis-DDP are electrophilic and may react at nucleophilic sites of DNA. That the intact parent drug does not react with DNA is indirectly suggested by the reduced affinity of cis-DDP for DNA when chloride concentrations are increased *in vitro* (Horacek and Drobnick, 1971), an effect presumably related to decreased formation of the aquated/hydroxylated platinum species.

Several lines of evidence indicate that the primary intracellular target of cis-DDP (or metabolite) is DNA. In bacteria, cis-DDP inhibits cell division (Rosenberg *et al.*, 1965), inactivates transforming DNA (Munchausen, 1974) and DNA containing bacteriophage (Shooter *et al.*, 1972), and reduces viability of DNA repair-deficient mutants (Beck and Brubaker, 1973). In addition, cis-DDP has been reported to be mutagenic (Beck and Brubaker, 1975), as indicated by its ability to induce base-pair substitutions (Monti-Bragadin *et al.*, 1975) and frame shift mutations (Andersen, 1979), In eukaryotic cells, cis-DDP selectively inhibits DNA synthesis in cultured human embryonic AV_3 cells (Harder and Rosenberg, 1970), Erlich ascites tumor cells (Howle and

Gale, 1970) and Chinese hamster cells (Van den Berg and Roberts, 1976). More platinum molecules are bound per molecule of DNA than per molecule of RNA or protein in HeLa cells treated with cis-DDP (Pascoe and Roberts, 1974), further indicating that DNA is a likely target for cis-DDP. In addition, cis-DDP also appears to be mutagenic in eukaryotic cells (Zwelling et al., 1979).

The binding of cis-DDP (or metabolite) to DNA may occur in several possible ways, including interstrand crosslinking (crosslinking of two bases on opposite DNA strands), mono- and bi-functional chelation to bases, and/or crosslinking between RNA and protein. Several studies have suggested that interstrand crosslinking or crosslinking to protein does not play a critical role in cis-DDP cytotoxicity (Pascoe and Roberts, 1974; Shooter et al., 1972). Rather, it has been proposed that intrastrand crosslinking represents a likely mechanism of platinum-DNA interaction. Indirect in vivo evidence supporting this concept has been provided by Roos (1977) who measured the binding of an intercalating agent (9-aminoacridine) (9AA) to DNA, with or without platinum pretreatment, as a means of assessing DNA-platinum interaction. Roos (1977) postulated that if cis-DDP (or metabolite) is avidly crosslinking two bases 3.4 Å apart on a single strand, then this intrabase separation will be preserved and prevent intercalation by 9AA. Conversely, interstrand crosslinking by cis-DDP (or metabolite) could still allow 9AA intercalation. Binding of 9AA to DNA, which increases base separation from 3.4 to 6-7 Å, is indeed decreased by prior reaction of DNA with active platinum compounds, indicating the likelihood of intrastrand crosslinking to DNA as a primary mode of interaction

by active platinum complexes. Unfortunately, the platinum complex under investigation was dichloro(ethylenediammine)platinum (II) and not cis-DDP. Although this compound and cis-DDP have similar anti-tumor activities it would have been of interest to examine the effects of cis-DDP on 9AA binding to DNA.

It is generally believed that active platinum complexes react primarily with purine bases, rather than sugar-phosphate moieties of DNA. Furthermore, it has been suggested that DNA intrastrand crosslinking may involve adjacent guanine bases and may represent an important lesion induced by cis-DDP (or metabolite) (Kelman et al., 1977). In this regard, several investigators have sought to explain the stereospecific anti-tumor activity of cis-DDP complexes by invoking a possible reaction site of DNA specific to the cis configuration. Guanine has been postulated as a likely reaction site since a cis geometry would facilitate formation of a closed ring bidentate chelate structure with the N7 and O6 sites of guanine. In contrast, the trans isomer may primarily form a monodentate ligand attachment at the N7 site of guanine; its geometry prohibiting formation of a closed ring chelate (Figure 3) (Goodgame et al., 1975; Macquet and Theophanides, 1976).

Chelation at the O6 site of guanine may be particularly significant to the stereospecific effects of DDP complexes on DNA integrity since other adducts (i.e., alkylated products) at this specific site have been causally linked to mutagenesis. On this basis, Rosenberg (1975; 1978b) has suggested that a "platinated" adduct, presumably at the O6 site of guanine, becomes fixed as a mutation, modifying the DNA

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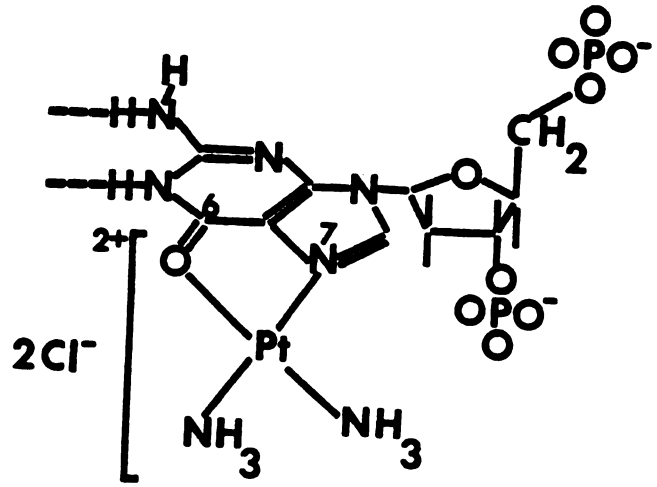
Figure 3. Proposed differences in binding of cis-DDP (top) and trans-DDP (bottom) to guanine. Binding of cis-DDP to guanine results in formation of a closed ring bidentate chelate structure with attachments at N7 and O6 sites of guanine. The geometry of trans-DDP prohibits formation of closed ring chelate.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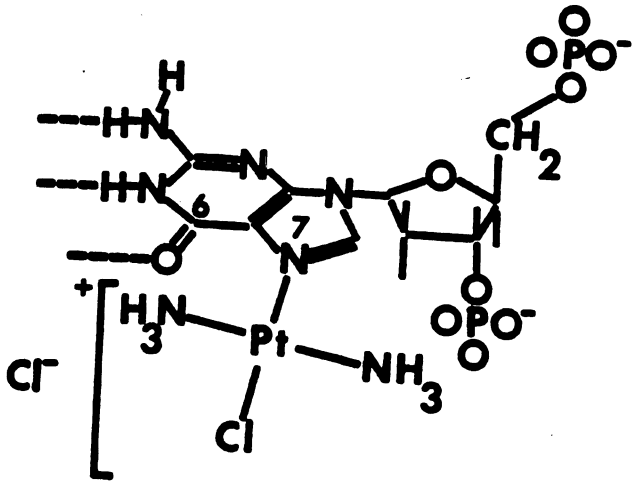
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Figure 3

template and resulting in selective inhibition of DNA replication by cis, but not trans, DDP. To account for the selective cytotoxicity to cancer cells, Rosenberg (1978b) has further postulated that the platinated adduct may be removed and repaired only by normal tissue; cancerous tissue may lack the necessary repair mechanisms. Along these lines, recent evidence suggests the presence of repair mechanisms for cis-DDP induced DNA damage: (1) cells harvested from Xeroderma pigmentosum patients are more sensitive to cytotoxicity of cis-DDP than normal fetal lung cells, indicating that deficient DNA repair mechanisms enhance cis-DDP cytotoxicity, (2) platinum is removed from DNA of exponentially growing Chinese hamster cells at a constant rate with a $t_{\frac{1}{2}}$ of 28 hrs and (3) there is a positive correlation between the extent of platinum binding to DNA and cell death, indicating that cell death may be a consequence of unexcised platinum lesions of DNA (Roberts and Fraval, 1980).

Another possible mechanism of the selective destruction of cancer cells has been proposed by Rosenberg (1972) as the enhanced antigenicity hypothesis. This hypothesis proposes that cis-DDP enhances immunological response of the host to cancer cells. Cancer cells produce new antigens at the cell surface and generate a host immune reaction resulting in destruction of these cells. According to Rosenberg (1972), these strong antigens of cancer cells are masked by weaker antigens, such as nucleic acids, and thereby escape host immune responses. Inasmuch as these cell surface nucleic acids are no longer apparent following cis-DDP treatment, it has been postulated that cis-DDP may disrupt antigen masking and expose the underlying stronger

antigens of cancer cells to stimulate an immune response. Direct evidence substantiating this attractive hypothesis is lacking.

Pharmacokinetics: Plasma Clearance and Protein Binding

Similar patterns of plasma clearance of cis-DDP have been observed. In all species studied to date, plasma concentrations following intravenous administration of cis-DDP decline in a biphasic manner, characterized by a rapid α $t_{\frac{1}{2}}$ and a more prolonged β $t_{\frac{1}{2}}$; however, the specific plasma clearance rates differ quantitatively according to species, dose, route of drug administration and time points analyzed. For example, the estimated $t_{\frac{1}{2}}$ of cis-DDP in the mouse is 1.6-5 hours (Hoeschele and Van Camp, 1972). In the rat, Litterst et al. (1976b) initially reported the α $t_{\frac{1}{2}}$ as 43 minutes and the β $t_{\frac{1}{2}}$ as 2 days, and reported in a later study, the α $t_{\frac{1}{2}}$ and β $t_{\frac{1}{2}}$ as 9.7 minutes and 35.7 hours, respectively, using more time points for their analysis (Litterst et al., 1979). In dogs, the initial rapid phase of plasma clearance of cis-DDP is similar to rats; however, the more prolonged β $t_{\frac{1}{2}}$ approximates 4-5 days in dogs (Litterst et al., 1976a). In humans, a biphasic mode of plasma clearance of platinum has also been observed, with an initial $t_{\frac{1}{2}}$ of 25-49 minutes and a secondary phase of 58-73 hours (DeConti et al., 1973). These findings are consistent with a recent clinical study by Gormely et al. (1979); however, in conducting experiments over a 21 day period, these investigators also observed a slow tertiary phase of plasma platinum clearance (Gormley et al., 1979). All of these studies, however, are difficult to interpret since only total platinum concentrations were determined and therefore may not reflect clearance of intact cis-DDP.

Plasma protein binding of platinum occurs within the first two hours of drug administration and may account for as much as 90% of administered dose (DeConti et al., 1973). Further analysis of platinum concentrations in blood have revealed its presence in at least three distinct pools: a free, protein bound and erythrocyte bound fraction (Manaka and Wolf, 1980). Inasmuch as total serum platinum concentrations apparently does not reflect active drug concentrations (Gormley et al., 1979), meaningful pharmacokinetic studies must discriminate between the disposition of free circulating drug from that of total drug (bound plus unbound) concentrations. Recent analytical developments utilizing centrifugal ultrafiltration and atomic absorption spectroscopy have provided the necessary means to distinguish free filterable platinum from protein bound species following cis-DDP administration (Bannister et al., 1977).

The kinetics of protein binding of cis-DDP are characterized by a first order reaction with a $t_{\frac{1}{2}}$ of 220 minutes, as indicated by the in vitro disappearance of platinum with time in ultrafiltrates of solutions containing human serum and cis-DDP (Gormley et al., 1979). Similar studies suggest that the rate of protein binding to platinum is too slow to be accounted for by affinity reactions (i.e., hydrogen bonding, van der Waals forces, etc.) (Long and Repta, 1981).

Although the plasma clearance of non-protein bound platinum appears to be biphasic in humans, the terminal half-lives for filterable platinum (32-53 minutes) are considerably shorter than those for total platinum (>67 hours) (Gormley et al., 1979; Himmelstein et al., 1981;

Patton et al., 1978). The nature of the protein-platinum reaction has been recently evaluated by equilibrium dialysis in which human plasma or 2% serum albumin in phosphate buffer was equilibrated with cis-DDP (Repta and Long, 1980). Theoretically, if protein-platinum reaction is reversible, then equilibrium would be established and characterized by equivalent platinum concentrations on either side of the dialysis membrane. Results, however, indicate no evidence of dialysis for periods of up to 24 hours, suggesting that the rate of release of platinum from the protein-platinum complex is slow and the reaction is not readily reversible (Repta and Long, 1980). Long and Repta (1981) therefore suggested that the protein-platinum reaction is probably one of covalent bond formation.

In most of these studies, analytical techniques are based upon the detection and quantification of the platinum atom; i.e., atomic absorption, X-ray fluorescence, neutron activation or isotopic assays. In all cases, it has been assumed that elemental or radiolabeled platinum represents the parent drug, thereby precluding the possibility of biotransformation. Thus, the relatively nonspecific nature of these techniques provides little information regarding the pharmacokinetics specific to the parent drug or its metabolic products. However, recently Chang et al. (1978) reported a method capable of specifically isolating and quantifying cis-DDP in the presence of other platinum containing species utilizing high performance liquid chromatography (HPLC) with a strong anion-exchange resin. Utilizing this technique, the pharmacokinetics of intact cis-DDP, total platinum and

non-protein bound platinum have been studied in patients receiving intravenous drug administration (Himmelstein et al., 1981). While total platinum concentrations in plasma appear to decline in a triphasic mode (with a terminal $t_{\frac{1}{2}}$ greater than 24 hours), both non-protein bound platinum and cis-DDP decline in a monophasic manner (with a terminal $t_{\frac{1}{2}}$ of 20-30 minutes). Furthermore, the ratio of cis-DDP to total non-protein bound platinum in plasma approximates 0.6-0.8 within 5 minutes of cis-DDP administration and remains in this range throughout the time course of sampling. The authors therefore suggest that cis-DDP is converted to other non-protein bound platinum forms only shortly after drug administration. Thus, measurement of total non-protein bound platinum probably provides a reasonable index of circulating cis-DDP concentrations, although overestimations are likely.

Using HPLC techniques, the half-life of cis-DDP in human plasma and plasma ultrafiltrates has been estimated as 1.5 and 2.2 hours, respectively (Long et al., 1980). The in vitro disappearance of cis-DDP from ultrafiltrates may be due to (1) association of cis-DDP with other low molecular weight plasma components or (2) biotransformation reactions. In support of the former, sulfhydryl groups of amino acids and/or peptides have been indicated as likely nucleophilic sites for cis-DDP reactivity (Repta and Long, 1980).

Pharmacokinetics: Excretion

cis-DDP and/or its related metabolic products are excreted primarily in the urine although a small amount of biliary and fecal excretion has

also been detected (DeSimone et al., 1979). Using radiolabeled platinum, DeConti et al. (1973) reported a bimodal pattern of urinary platinum excretion in humans, characterized by a rapid initial phase and a more prolonged and incomplete later phase. The amount of cis-DDP recovered as platinum in the urine has been estimated as 35% at 4 hours, 40% at 24 hours and 50% four days following intravenous drug administration (Lagasse et al., 1981). In rats, ,75% of the administered dose is recovered as platinum in urine 30 days following drug administration (Litterst et al., 1979). However, these studies suffer from non-specific determination of platinum and provide little information regarding the chemical forms of cis-DDP excreted. Recently at least two platinum species in the urine have been identified using ion exchange chromatography: a water elutable (presumably cis-DDP) and HCl elutable (presumably aquated/hydroxylated complex) fraction (LeRoy et al., 1980). During cis-DDP infusion to patients, a rapid urinary excretion of both of these species appears in proportions similar to the composition of drug infusate (LeRoy et al., 1980). Following drug treatment, urinary excretion of the water elutable fraction decreases while that of the acid-elutable fraction increases with time. These authors suggested that the quantitative aspects of urinary platinum excretion may depend on factors such as urinary concentration of chloride and/or pH.

Recently, the renal handling of cis-DDP has been investigated in patients receiving a 24 hour drug infusion. Using plasma ultrafiltrate and atomic absorption techniques, it has been reported that free platinum clearance exceeds creatinine clearance, suggesting tubular

secretion of cis-DDP (or metabolite) (Jacobs et al., 1980). Although these results have been confirmed by a recent preliminary report (Safirstein et al., 1981a), more definitive information regarding the nature of the platinum species excreted in the urine and their respective handling by the kidney is needed.

Pharmacokinetics: Tissue Distribution

Studies examining the in vivo disposition of platinum following drug administration indicate an initial distribution to nearly all tissues, followed by a specific accumulation in kidney, liver, muscle and skin (Litterst et al., 1979). Thus far, similar patterns of drug distribution have been documented in all species studied including mice (Hoeschele and Van Camp, 1972; Lange et al., 1972), rats (DeSimone et al., 1979; Litterst et al., 1976b), rabbits (Lange et al., 1972), dogs (LeRoy et al., 1979; Litterst et al., 1976) and dogfish sharks (Litterst et al., 1979). A consistent finding of these studies is the localization and prolonged retention of platinum in the kidney ($t_{\frac{1}{2}}$ = 50 hours) and liver ($t_{\frac{1}{2}}$ = 32 hours) (Choie et al., 1980). When tissue mass is taken into account the absolute amount of platinum in the rat is greatest in skin, bone, muscle and liver for up to three days following drug treatment (Wolf and Manaka, 1976). In contrast, very low concentrations of platinum are present in brain tissue (Hoeschele and Van Camp, 1972; Lange et al., 1972; Litterst et al., 1976b); although recently it has been reported that brain concentrations of platinum increase two-to-three fold following the first day of drug treatment in dogfish sharks (Litterst et al., 1979).

Interestingly, the distribution of cis-DDP to target organs of anti-tumor activity does not reveal any consistent correlation; that is, although high platinum concentrations are detected in ovarian and uterine tissue, very low concentrations are reported in the testes (Litterst et al., 1976b). Comparisons of platinum tissue distribution in tumored versus non-tumored animals have also been investigated in an attempt to account for the preferential cytotoxicity of cis-DDP to cancer cells. Although there appears to be no specific uptake of platinum by tumor tissue (Toth-Allen, 1970), consistently higher platinum concentrations have been reported in tissues of tumored, compared to non-tumored, mice 108 hours after drug administration, an effect which may be attributable to reduced drug excretion by tumored animals (Hoeschele and Van Camp, 1972).

The subcellular distribution of cis-DDP in HeLa cells appears to be greatest in the nuclear fraction whereas the cytoplasm contains little or no platinum (Kahn and Sadler, 1978). Although there is a paucity of information regarding subcellular platinum distribution following in vivo drug administration, Choie et al. (1980) recently reported that specific platinum concentrations (μg Pt/mg protein) in kidney nuclei and microsomes are significantly greater than those in kidney mitochondria, plasma membrane or cytosol. In contrast to the kidney, no specific subcellular accumulation of platinum is observed in hepatic tissue (Choie et al., 1980). The lack of association of platinum with hepatic microsomes is consistent with the absence of any

effect of cis-DDP on the activities of hepatic microsomal drug metabolizing enzymes following single drug administration (Litterst *et al.*, 1979.

Pharmacokinetics: Biotransformation

Biotransformation of cis-DDP is suggested by the *in vitro* disappearance of cis-DDP and concomitant appearance of other platinum compounds in plasma and plasma ultrafiltrates. Utilizing gel filtration chromatography techniques, at least seven platinum species have been identified in plasma ultrafiltrates 12 hours following addition of cis-DDP (Long and Repta, 1980). Further characterization by X-ray fluorescence indicates the presence of both sulfur and platinum in approximately equimolar concentrations. Molecular weights of the four major species are estimated as 600, 500, 465 and 440 daltons (Repta and Long, 1980).

In vivo studies indicate that the chromatographic profile of platinum species in plasma ultrafiltrates and urine from a patient receiving cis-DDP are markedly different than that observed *in vitro* (Repta and Long, 1980). However, a peak with an estimated molecular weight of 440 is evident in both ultrafiltrate and urine and corresponds closely to *in vitro* results. The apparently low molecular weights of these platinum compounds coupled with the presence of sulfur suggests that cis-DDP probably reacts non-enzymatically with low molecular weight proteins, peptides and/or amino acids. To date, studies on the *in vivo* metabolism of cis-DDP in both extracellular and intracellular compartments are

limited and require further attention if the physiological disposition and molecular actions of cis-DDP are to be understood.

Toxic Effects of cis-DDP: Kidney

Clinical use of cis-DDP is primarily limited by its dose-related and cumulative renal toxicity, an effect which is well-documented in all species studied to date including mice (Leonard et al., 1971; Schaeppi et al., 1973), rats (Choie et al., 1980; Kociba and Sleight, 1971; Safir-

stein et al., 1981b; Ward and Fauvie, 1976), dogs (Cvitkovic et al., 1977; Litterst et al., 1976a; Schaeppi et al., 1973), monkeys (Leonard et al., 1971; Schaeppi et al., 1973) and humans (Dentino et al., 1978; Gonzalez-Vitale et al., 1977). In laboratory animals, cis-DDP nephrotoxicity is characterized histologically by acute tubular necrosis in the corticomedullary region (Choie et al., 1981; Dobyan et al., 1980; Ward and Fauvie, 1976). Although there is uniform agreement that cis-DDP nephrotoxicity primarily involves degenerative changes in the proximal tubule, there are conflicting reports regarding its effects on the distal tubules, with some studies reporting either moderate to severe (Aggarwal et al., 1980; Choie et al., 1981) or an absence of (Dobyan et al., 1980; Lehane et al., 1979; Safirstein et al., 1981b) damage to distal tubules of rat kidneys. In humans, reports of cis-DDP nephropathy are limited; however, Gonzalez-Vitale and coworkers (1977) reported focal acute tubular necrosis, affecting primarily the distal convoluted and collecting tubules at autopsy. To date, glomerular or vascular lesions following cis-DDP treatment have not been detected.

The time course of cis-DDP nephropathy in laboratory animals is characterized by degenerative changes in the proximal tubule as early as 1-2 days following treatment and consists of cytoplasmic vacuolization, tubular dilatation, pyknotic nuclei and hydropic degeneration (Choie et al., 1980; Dobyan et al., 1980; Ward and Fauvie, 1976). By days 3-5 pathologic changes are the most profound and are characterized by widespread tubular necrosis of the corticomedullary region, predominantly in the third segment (S_3) or straight portion of the proximal tubule (pars recta) (Choie et al., 1980; Dobyan et al., 1980; Lehane et al., 1979; Safirstein et al., 1981b; Ward and Fauvie, 1976). Electron microscopic studies reveal several ultrastructural changes in the pars recta including: profound thinning or focal loss of brush border, cellular swelling, condensation of nuclear chromatin, cytoplasmic vacuolization, rounded mitochondria with swollen cristae, dissociation of mitochondria from basal infoldings, loss of basal infoldings and an increased number and size of pinocytotic vesicles and lysosomal bodies in the apical region bordering the lumen (Aggarwal et al., 1980; Dobyan et al., 1980). Animals surviving cis-DDP nephrotoxicity demonstrate renal tubular regeneration as indicated by enlarged nuclei and mitotic figures. However, the presence of necrotic debris in tubular lumen coupled with persistent tubular damage for six months following single administration of cis-DDP (Dobyan et al., 1980) suggests incomplete recovery. In addition, chronic treatment with cis-DDP may result in cyst formation (Dobyan et al., 1981), and interstitial fibrosis and thickening of tubular basement membranes (Choie et al., 1980), causing irreversible renal damage.

Functional correlates of cis-DDP nephrotoxicity include transient elevations in blood urea nitrogen (BUN) and serum creatinine concentrations (Gonzalez-Vitale et al., 1977; Kociba and Sleight, 1971; Ward and Fauvie, 1976) and polyuria despite diminished glomerular filtration rate (GFR) (Safirstein et al., 1981b). Micropuncture studies indicate that cis-DDP treatment compromises superficial single nephron GFR (SNGFR); however, the magnitude of its reduction is insufficient to account for the decline in whole kidney GFR, suggesting that cis-DDP profoundly affects GFR in juxtamedullary nephrons (Safirstein et al., 1981b). A defect in concentrating ability is also a striking feature of cis-DDP nephrotoxicity and appears to be related to a diminished corticopapillary solute gradient, an effect associated with a failure to recycle urea (Safirstein et al., 1981b).

The mechanisms underlying cis-DDP nephrotoxicity, and in particular the profound necrosis of the pars recta, remain unclear. Choie and coworkers (1980) have postulated that the localization and severity of tubular necrosis following cis-DDP treatment is related to the regional platinum distribution within the kidney. This postulate is supported by the observed concentration gradient of platinum in the kidney with the highest concentration in the corticomedullary junction and the lowest platinum concentration in medullary tissue, an effect correlating with the localization of tubular necrosis (Choie et al., 1980). The mechanisms responsible for the selective accumulation of platinum in corticomedullary tissue are not well-identified. However, it is known that the pars recta is a major site of active tubular secretion, a process which initially involves active transport from

peritubular fluid into proximal tubules, resulting in intracellular accumulation of the transported molecule. On this basis, it has been postulated that cis-DDP (or metabolite) may be transported by a similar mechanism and may account for selective intracellular accumulation of platinum in the pars recta (Dobyan et al., 1980). Although cis-DDP appears to undergo renal tubular secretion (Jacobs et al., 1980; Safirstein et al., 1981a), characterization of this transport mechanism and direct evidence linking it to intracellular platinum accumulation in the pars recta is lacking.

An important aspect of cis-DDP nephrotoxicity which has not been adequately addressed is the chemical nature of the immediate nephrotoxicant. Inasmuch as the gross characteristics of cis-DDP nephropathy appear similar to the histopathological changes observed following administration of other heavy metals, particularly mercury, it has been presumed that the nephrotoxicity of cis-DDP is related to the toxicity of the platinum atom (Choie et al., 1980; Madias and Harrington, 1978). Characteristics common to both cis-DDP and mercuric-chloride nephrotoxicity include: (1) acute tubular necrosis affecting primarily the pars recta (Dobyan et al., 1981), (2) increased size and number of renal lysosomes (Aggarwal et al., 1980), and (3) depletion of protein-bound SH groups (Levi et al., 1980). However, several lines of evidence suggest that the differences between cis-DDP nephrotoxicity and mercuric chloride toxicity are quite distinct:

(1) Time course and development. The earliest detectable changes in the kidney appear several days following cis-DDP treatment. Although

GFR is compromised 3, 7, 14 and 22-30 days following a single administration of cis-DDP (5 mg/kg) to rats, no differences are observed on days 1 and 2 (Safirstein et al., 1981). Similarly, changes in urinary composition and volume are not evident until several days following treatment (Safirstein et al., 1981). In contrast, alterations in renal function following administration of even a small dose of mercuric chloride are rapidly induced within 24 hours (Haagsma and Pound, 1979). Thus, the delayed appearance of cis-DDP nephrotoxicity suggests that its underlying mechanisms probably differ from mercuric chloride and may be related to the time needed for biotransformation. In addition, tubular regeneration is complete 9-14 days following mercuric chloride (Haagsma and Pound, 1979), but not cis-DDP (Choie et al., 1980; Dobyan et al., 1981), treatment, further suggesting differences in the time course of kidney damage and repair between these compounds.

(2) Chelation therapy. Mercuric chloride nephrotoxicity is often reversible following treatment with sulfhydryl reacting chelating agents, i.e., cysteamine, penicillamine and N-acetylcysteine. In contrast, cis-DDP nephrotoxicity is not reduced by metal chelators (Graziano et al., 1981), suggesting a dissociation between the nephrotoxic actions and molecular reactivity of cis-DDP and mercury.

Furthermore, several lines of evidence suggest that cis-DDP nephrotoxicity may not be solely attributable to the platinum atom:

(1) Stereospecificity of cis-DDP. Although cis-DDP and trans-DDP result in similar renal concentrations of platinum (Van Camp and

Hoeschele, 1972), the trans isomer does not induce renal toxicity (Leonard et al., 1971; Van den Berg et al., 1981), indicating that the geometry of these complexes probably plays a crucial role in producing nephrotoxicity and that the presence of the platinum moiety alone may not be sufficient for inducing nephrotoxicity.

(2) Structure-activity relationships. Modification of ligands of DDP complex alters the nephrotoxicity. The following chemical properties appear to be related to nephrotoxicity: (a) presence of N-H in coordinating amines, (b) absence of bulky alkyl groups in coordinating amines, and (c) chelate ring size, with smaller ring associated with increased toxicity (Broomhead et al., 1980). Thus, modulation of nephrotoxicity by altering the ligands of platinum complexes indicates that toxicity is not solely related to the platinum atom.

Inasmuch as the immediate nephrotoxicant of cis-DDP has not been identified as of yet, the molecular mechanisms of cis-DDP nephrotoxicity remain poorly understood. Since cis-DDP (or metabolite) is known to react with nuclear DNA, it has been suggested that its nephrotoxicity may result from a similar mechanism. The selective accumulation of platinum in renal nuclei (Choie et al., 1980) may be particularly significant in this regard. Other mechanisms of renal toxicity which have been suggested, but not adequately tested, include depletion of renal sulfhydryl groups (Dobyan et al., 1980) inhibition of renal Na,K-ATPase (Guarino et al., 1979), and activation of renin-angiotensin system (Madias and Harrington, 1978).

Modulation of Kidney Toxicity

Since nephrotoxicity limits the clinical use of cis-DDP, numerous attempts have been made to reduce kidney damage and increase the therapeutic index of this drug. Cvitkovic et al. (1977) demonstrated that vigorous intravenous hydration before, during and immediately after cis-DDP administration reduces the incidence and severity of renal toxicity in dogs. Similarly, mannitol (10 g/hr) in conjunction with hydration (0.45% saline, 200 ml/hr) for six hours after cis-DDP administration significantly improves the therapeutic index of cis-DDP (Hayes et al., 1977). Although these manipulations reduce cis-DDP nephrotoxicity, as indicated by the prevented rise in BUN concentrations, drug half-life and plasma clearance and tissue distribution of platinum are unaltered (Pera et al., 1979; DeSimone et al., 1979). Furthermore, the anti-tumor properties of cis-DDP are not compromised (Hayes et al., 1977; Pera et al., 1979). The reduction in cis-DDP nephrotoxicity associated with mannitol pretreatment appears to be related to reduced urinary concentration of platinum, although total urinary platinum excretion is not affected (Pera and Harder, 1979). Histopathological evaluation of kidneys on days 1-4 indicate, however, an equivalent degree of proximal tubular necrosis in rats treated with cis-DDP alone or in combination with mannitol. Thereafter, a trend developed toward less persistence of histopathological damage in mannitol treated groups (Pera et al., 1979).

Other attempts to modify cis-DDP nephrotoxicity include pretreatment with probenecid (Ross and Gale, 1979), thiosulfate (Howell and

Taetle, 1980), superoxide dismutase (McGinness et al., 1978), furosemide (Ward et al., 1977) and chelating agents (Graziano et al., 1981). Recently, Litterst (1981) has reported that alterations in the vehicle concentration of NaCl influence renal toxicity. Specifically, nephrotoxicity is increased when cis-DDP is prepared in distilled water. Conversely, cis-DDP nephrotoxicity is reduced when prepared in increasing concentrations of NaCl. Furthermore, kidney platinum concentrations and plasma protein binding of platinum are both markedly elevated following administration of drug prepared in distilled water, compared to 0.9% or 4.5% NaCl drug solutions. These results therefore suggest that cis-DDP prepared in a water vehicle facilitates the formation of an aquated/hydroxylated platinum species, resulting in enhanced plasma protein and tissue binding. Although efforts to modify cis-DDP nephrotoxicity have been somewhat successful, complete protection from nephrotoxicity will only be feasible when the mechanism of cis-DDP nephrotoxicity is more fully understood.

Other Toxic Effects

In addition to kidney toxicity, gastrointestinal disturbances, characterized by nausea, vomiting and diarrhea, also represent major clinical problems associated with cis-DDP therapy. Although the pathogenesis of these gastrointestinal disturbances has not been defined in humans, several reports indicate histological damage of the intestinal tract of laboratory animals. Kociba and Sleight (1971) reported edematous villi of intestinal mucosa as early as one day following cis-DDP treatment. On days 3-4 histopathological lesions are the most

severe, coinciding with development of fluid distension and diarrhea, and are characterized by denuded mucosal surface and a cyst-like appearance of the crypts of Lieberkuhn. The intestinal epithelium of surviving rats demonstrate increased mitotic activity of cells lining the crypts, indicating regeneration. A more recent study has confirmed and extended these observations, indicating that cellular necrosis is most severe in the ileum, followed by the jejunum and duodenum, while neither the stomach nor colon are markedly affected (Choie et al., 1981a). Inasmuch as tissue concentrations of platinum are comparable in all segments of the GI tract, the effect of cis-DDP on GI mucosa may be related to other factors, e.g., pH (Choie et al., 1981a). Pancreatitis has also been observed in cis-DDP treated dogs (Schaeppi et al., 1973) and there has been one report of liver toxicity in a treated patient (Cavalli et al., 1978).

Myelosuppression, characterized by leukopenia, thrombocytopenia and anemia, is also observed following cis-DDP treatment (Kociba and Sleight, 1971 ; Von Hoff et al., 1979). Other toxic side effects of cis-DDP include ototoxicity (tinnitus, hearing loss) (Helson et al., 1978; Von Hoff et al., 1979), allergic reactions (eczema, anaphylactic reactions) and peripheral neuropathy (Von Hoff et al., 1979). A potentially serious toxic effect of cis-DDP is drug induced hypomagnesemia. A phase II prospective clinical trial has indicated the development of hypomagnesemia in 23 of 44 patients receiving cis-DDP (Schilsky et al., 1979), an effect which may be attributable to impaired tubular reabsorption of magnesium, resulting in inappropriate urinary losses.

Hypocalcemia has also been observed in 4 of 8 patients (Hayes et al., 1979).

Pharmacology and Toxicology of Other Platinum Compounds

The increasing utility of platinum as the active component of the automotive catalytic converters has stimulated interest in its toxicity following inhalation. Moore and coworkers (1975a,c) reported that immediately following inhalation of the soluble platinum salts, ^{191}Pt is distributed in the gastrointestinal and respiratory tract. In addition, kidney and bone contain the greatest amount of radioactivity. Intravenous administration of platinum to rats results in significant platinum accumulation in kidney, liver, spleen, adrenal gland and pancreas (Moore et al., 1975a,b,c). Following a single oral dose, almost all of the ^{191}Pt is excreted in the feces due to malabsorption; whereas, similar quantities are excreted in both urine and feces following intravenous administration.

Information regarding the toxicology of platinum salts is extremely limited. However, it has been reported that workers chronically exposed to platinum metal salts in refineries are subject to platinosis, a condition characterized by dermatitis, eczema, skin ulcerations and respiratory distress (LeRoy, 1975).

ENDOCRINE REGULATION OF GLUCOSE HOMEOSTASIS

General Aspects of Biological Actions of Insulin and Glucagon

Despite differing nutritional states, blood glucose concentrations in normal humans are generally maintained within the narrow range of

3-7 mM. Such stringent regulation of blood glucose is probably reflective of the importance of glucose as a primary respiratory fuel, particularly by the central nervous system. Thus, if blood glucose concentrations are to remain fairly constant, both influx and efflux of glucose from extracellular fluid (ECF) must be balanced. In this regard, the liver plays a major regulatory role by controlling glucose fluxes in response to variations in dietary intake and to variations in fuel homeostasis. For example, in response to low glucose concentrations in portal venous blood net glucose production and released into ECF is increased. Conversely, in response to increased glucose concentrations in portal vein, net uptake and metabolism of glucose is increased. In this way, the liver is able to exert tight regulation of peripheral blood glucose concentrations.

The intrinsic ability of the liver to respond appropriately to glycemic stimuli is governed, in part, by the biological actions of insulin and glucagon perfusing the liver. Both insulin and glucagon are synthesized in pancreatic islets; the insulin-containing beta cells form more than 60% of the islet cell population and are arranged in a relatively homogeneous central mass while the glucagon containing alpha cells are situated at the periphery of the islet and comprise approximately 30% of islet cells. Somatostatin containing delta cells form the remaining 10% of islet cell population. Both insulin (MW ≈6000) and glucagon (MW ≈3485) are proteolytic products of larger precursor molecules, i.e., proinsulin (MW ≈9000) and proglucagon (MW ≈9000-18,000), respectively.

Proinsulin, synthesized by ribosomes associated with the rough endoplasmic reticulum of beta cells, is translocated to the Golgi apparatus by an energy dependent system where hydrolytic cleavage yields insulin and C-peptide. These products, packaged into granules, migrate to the periphery of the cell, fuse with the plasma membrane and are ultimately extruded by an exocytotic process into ECF. The normal secretory products of the beta cell, therefore, include insulin, an equimolar concentration of C-peptide and a small amount (≈5%) of unconverted proinsulin. Once secreted, the initial action of insulin depends upon specific receptor binding in the plasma membrane of target tissues. It is generally believed that following binding, the insulin-receptor complex generates one or more signals and may include changes in conformation of plasma membrane, ion flux (Na^{+}, Ca^{+2}, Mg^{+2}) and/or nucleotides (ATP, cyclic AMP) (Czech, 1981). These signals or second messengers then interact with a variety of effector units which ultimately mediate the host of biological actions attributable to insulin. The cardinal feature of insulin action is the promotion of nutrient storage and/or utilization. With respect to carbohydrate metabolism, insulin stimulated glucose utilization is characterized by increased tissue uptake and metabolism of glucose (glycolysis) while storage of carbohydrates is effected by increased glycogen synthesis. In contrast, the effects of glucagon on carbohydrate metabolism are characterized by mobilizing nutrients from storage depots and are mediated by the action of cyclic AMP. The biological effects of glucagon are thus antagonistic to those of insulin and include increased glycogenolysis and gluconeogenesis.

The molar ratio of insulin to glucagon (I/G) has been suggested to provide a more accurate index of net biological action than the absolute level of either hormone alone (Unger, 1971). Thus, an elevated I/G would promote glycogen storage and inhibit endogenous glucose production. Conversely, a reduced I/G would favor mobilization of stored glycogen and increase gluconeogenesis. For example, in fasting animals, the concentration of glucagon, relative to insulin, must be sufficiently high to maintain hepatic fuel production to meet the fuel needs of the organism. On the other hand, when the need for endogenous glucose production is diminished by glucose ingestion, I/G increases markedly and promotes carbohydrate storage and/or utilization (Unger, 1971). In this way, the maintenance of normoglycemia is achieved through a "push-pull" system of two tightly coordinated biological antagonists, insulin and glucagon.

EFFECTS OF HEAVY METALS ON CARBOHYDRATE METABOLISM

Several divalent metal ions, including cadmium, nickel, cobalt, copper, zinc and mercury affect carbohydrate metabolism (Horak and Sunderman, 1975b). Of these metals, cadmium and nickel have been studied the most extensively. Ghafghazi and Mennear (1973) were the first to demonstrate that administration of a single dose of cadmium (6 mg/kg, i.p.) impairs glucose tolerance in mice. Glucose intolerance is observed 1 hour, but not 24 hours, following cadmium administration. In addition, these authors reported a reduced immunoreactive insulin response to glucose stimulation in cadmium treated animals. In

contrast to these acute effects, repeated injections of cadmium (4 mg/kg/day x 14 days) does not impair glucose tolerance (Ghafghazi and Mennear, 1973). The authors attributed the absence of glucose intolerance to the possible induction of metallothionein synthesis, which presumably sequesters the cadmium ion. To more clearly define the role of the pancreas in cadmium induced glucose intolerance, Ghafghazi and Mennear (1975) utilized the isolated perfused rat pancreas to demonstrate a direct inhibitory effect of cadmium on pancreatic insulin secretory activity. The inhibition of insulin secretion by cadmium appears to be nonspecific, immediate in onset and is not reversed by washout of the pancreas with perfusion medium. However, perfusion of cadmium treated pancreata with both glucose and theophylline results in a partial restoration of normal insulin secretion. The partial restoration of insulin secretion in cadmium treated pancreata by theophylline may be due to the effects of theophylline on intracellular calcium flux and/or on pancreatic islet cyclic AMP (cAMP) metabolism (Ghafghazi and Mennear, 1975).

Administration of cadmium chloride (1 mg/kg/day x 21 days) has also been reported to markedly elevate hepatic cAMP levels (Merali et al., 1975). Chronic treatment with cadmium chloride (0.25 mg/kg/day x 45 days) markedly elevates the activities of key gluconeogenic enzymes (Merali et al., 1975). Single administration of cadmium (60 mg/kg) does not however affect gluconeogenic enzyme activity one hour following treatment, although elevated blood glucose and hepatic cAMP levels are evident at this time (Singhal et al., 1976). Since alterations in

cAMP precede elevations in gluconeogenic enzyme activity, it has been proposed that the primary lesion mediating cadmium induced hyperglycemia involves altered cAMP metabolism (Singhal et al., 1976).

The effects of cadmium on adrenal catecholamine metabolism have also been investigated. Daily intraperitoneal injections of cadmium chloride (1 mg/kg/day x 45 days) significantly augments adrenal weight and adrenal norepinephrine and epinephrine concentrations (Rastogi and Singhal, 1975). Inasmuch as catecholamines may inhibit insulin secretion, the reduced insulin secretory response following cadmium treatment may be secondary to the effects of catecholamines. To further examine the influence of the adrenal glands on cadmium induced hyperglycemia, Ghafghazi and Mennear (1973) measured blood glucose levels in adrenalectomized mice treated with a single dose of cadmium. Since cadmium induced hyperglycemia was abolished in adrenalectomized mice, the authors concluded that the hyperglycemic response to cadmium may be adrenal-mediated.

Nickel (II) also induces a transient elevation in plasma glucose (Horak and Sunderman, 1975; Clary, 1975). Peak plasma glucose levels are observed 0.5 hours following intraperitoneal injection of nickel (II) as either $NiCl_2$ or $NiSO_4$ and is associated with elevated levels of plasma glucagon in rats (Horak and Sunderman, 1975), suggesting that hyperglucagonemia may be responsible for nickel induced hyperglycemia. Plasma concentrations of glucagon and glucose returned to control values within 2-4 hours following nickel administration. Horak and Sunderman (1975) further observed that the hyperglycemic response to

nickel was suppressed, but not completely abolished, by adrenalectomy. To date, the exact mechanism(s) of nickel induced hyperglycemia remain(s) poorly defined.

RATIONALE

Recent investigations have suggested that several divalent metal ions, particularly cadmium and nickel, markedly affect carbohydrate metabolism. Therefore, a divalent platinum compound, such as cis-DDP, may similarly alter carbohydrate metabolism. Several lines of evidence suggest that cis-DDP may affect glucose homeostasis: (1) cis-DDP treatment in rats results in random hyperglycemia (Kociba and Sleight, 1971) and (2) adrenal hyperplasia in mice (Toth-Allen, 1970) and pancreatic necrosis in dogs (Schaeppi et al., 1973) have been observed following cis-DDP treatment. This study was therefore designed to characterize the effects of cis-DDP and other divalent platinum compounds on carbohydrate metabolism and to elucidate the underlying biochemical and endocrine mechanisms.

MATERIALS AND METHODS

GENERAL

Animals and Diet

Adult male Fischer-344 (F-344) rats, weighing 175-200 g, were purchased from either Charles River (Boston, MA) or Harlan Industries (Indianapolis, IN). All animals were housed in sanitary, ventilated animal rooms with controlled humidity, temperature, and 12-12 light-dark cycle for the duration of the experiments. A minimum of four days prior to experimental manipulation was allowed for acclimation to animal quarters. Unless indicated otherwise, all animals were offered a closed formula laboratory chow (Wayne Lab Blox, Allied Mills, Inc., Chicago, IL) and distilled water ad-libitum.

Platinum Treatment

The platinum compounds tested were cis-DDP and trans-DDP (kindly provided by Dr. Barnett Rosenberg, Department of Biophysics, Michigan State University, E. Lansing, MI) and ammonium tetrachloroplatinate [$(NH_4)_2PtCl_4$] (Aldrich Chemical Company, Milwaukee, WI). All agents, prepared in 0.9% NaCl, were administered intravenously as a single bolus injection. Rats were lightly anesthetized with ether and received platinum or a saline vehicle via a jugular vein. The relative insolubility of the DDP complexes in concentrations exceeding 1 mg/ml necessitated a volume injection of 16 ml/kg. Therefore, all treatment groups,

including controls receiving saline, were administered this volume unless indicated otherwise. Twenty-four hours prior to treatment was designated as day 0.

Glucose Tolerance Tests

Animals were fasted 4-5 hours prior to glucose tolerance tests. Glucose tolerance was evaluated by serially sampling blood (approximately 400 µl) before and 15, 30, 60 and 120 minutes following a glucose load (2 g/kg, i.p., 8 ml/kg). Venous blood was sampled by orbital sinus puncture in unanesthetized animals and was collected in test tubes containing EDTA (14 mg/ml blood) as an anticoagulant and 1% NaF to inhibit glycolysis. Following centrifugation at 2000 x g for 10 minutes, the plasma fraction was separated, frozen at 0°, and later analyzed for glucose using glucose oxidase, peroxidase and o-dianisidine as reagents (Sigma Chemical Company, St. Louis, MO).

Insulin and Glucagon Determination

For measurement of plasma immunoreactive insulin (IRI) and glucagon (IRG), blood was collected in test tubes containing EDTA (14 mg/ml blood) as an anticoagulant, and Traysylol (aprotinin) (FBA Pharmaceutical, New York, NY) (1000 kallikrein inactivator units/ml blood) to inhibit the proteolytic degradation of glucagon. Both IRI and IRG assays were conducted under the supervision of Dr. Ronald Gingerich (Department of Pediatrics, Washington University, St. Louis, MO) using a double antibody system.

IRI was assayed according to the method of Morgan and Lazarow (1963) and is based on a two step reaction involving:

(1) Incubating sample, ^{125}I-insulin (300-350 μCi/μg), and anti-insulin plasma obtained from guinea pigs, in a 0.05 M sodium, potassium phosphate buffer containing 0.025 M EDTA and 1% bovine serum albumin. Incubations were conducted overnight at 4°C, allowing adequate time for equilibration and formation of a soluble insulin-antibody complex.

(2) Following overnight incubation, anti-guinea pig plasma, obtained from rabbits, was added to samples and were incubated at 4° for 2.5 hours. Addition of anti-guinea pig plasma results in precipitation of the once soluble insulin-antibody complex. Samples were then centrifuged and the precipitate quantified for ^{125}I activity utilizing gamma counting techniques. The percent of radioactivity in the precipitate is inversely proportional to the concentration of IRI in the plasma samples. Calibration curves are based on porcine insulin standards. IRI concentrations are expressed as μUnits/ml.

IRG was assayed according to the method of Leichter et al. (1975) and was conducted in a similar manner to the IRI assay with the following exceptions:

(1) Incubation reaction included plasma sample and anti-glucagon plasma obtained from guinea pigs. Samples were incubated for three days at 4° in a 0.2 M glycine buffer containing 10% Traysylol and 1% bovine serum albumin. Following incubation, ^{125}I-glucagon (400-445 μCi/μg) was added to samples and incubated at 4° overnight.

(2) Following overnight incubation, anti-guinea pig plasma obtained from goats, was added to samples, incubated for 2.5 hours at 4° and centrifuged. ^{125}I activity was determined in the precipitate using gamma counting techniques. Calibration curves are based on porcine glucagon standards. IRG concentrations are expressed as pg/ml.

SPECIFIC PROTOCOLS

Effect of Platinum on Serum Glucose in Nonfasting Animals

Male F-344 rats were administered a single intravenous dose of 0, 2.5, 5, 7.5 or 15 mg/kg cis-DDP or trans-DDP. Ammonium tetrachloroplatinate was administered in a similar manner in doses equimolar in platinum concentration to those used in the DDP studies, yielding final doses of 0, 3, 6, 9 or 18 mg/kg of the platinum salt. Since preliminary studies indicated a dose-related anorexia associated with cis-DDP treatment, a group of controls was pair-fed on a daily basis to cis-DDP treated animals at each dose studied to correct for the metabolic effects of reduced food intake. Immediately before and 1, 2, 4, 7 and 14 days following platinum treatment, animals were lightly anesthetized with ether and serum obtained from orbital sinus blood was analyzed for glucose as previously described.

Effect of cis-DDP on Serum Glucose in Fasting Animals

Male F-344 rats were administered a single bolus injection of 0, 2.5, 5, 7.5 or 10 mg/kg cis-DDP (5 ml/kg). cis-DDP (1.5 mg/ml) was prepared in 0.9% NaCl and required gentle heating prior to injection. Animals were fasted 4-5 hours prior to each blood sampling. Orbital blood was sampled in anesthetized animals every 12 hours for the first five days

and then daily from days 5 to 7. Serum was analyzed for glucose as previously described.

Effect of cis-DDP on Glucose Tolerance

Rats were treated with a single intravenous dose of 0, 2.5 or 7.5 mg/kg cis-DDP (5 ml/kg). A pair-fed control group, restricted to the amount of food consumed by the 7.5 mg/kg group, was also studied. Since meal-feeding is known to alter carbohydrate tolerance, an attempt was made to simulate ad-libitum consumption in pair-fed animals by offering the allotted food over several time points during the day. Two days following treatment, glucose tolerance was measured by collecting serial blood samples before and after an intraperitoneal glucose load as previously described. Plasma from three identically treated rats was pooled, frozen at 0° and later assayed for glucose, IRI and IRG concentrations.

Effect of Platinum on Glucose Tolerance: Time Course and Dose Response

Glucose tolerance was evaluated 2, 4, 7 and 14 days following treatment with cis-DDP (0, 2.5 or 5 mg/kg), trans-DDP (0, 5, 7.5 or 15 mg/kg), or ammonium tetrachloroplatinate (0, 6 or 18 mg/kg). Plasma from three identically treated rats was pooled and analyzed for glucose. In specific incidences, plasma was also analyzed for IRI and IRG.

Effect of Platinum on Selected Organ Weights and Serum Amylase Activity

Following completion of glucose tolerance tests on day 4, and in some specific incidences on day 14, animals treated with cis-DDP, trans-DDP or ammonium tetrachloroplatinate in above experiments were sacrificed.

Liver, kidneys and adrenal glands were rapidly excised and wet weight determined.

On day 4, rats treated with cis-DDP were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and blood sampled from the abdominal aorta. Serum was colorimetrically analyzed for amylase activity using a standardized extract of corn starch as substrate (Sigma Chemical Company, St. Louis, MO). Results are expressed as Somogyi Units/dl. A Somoygi unit is defined as that amount of amylase causing formation of reducing power equivalent to 1 mg glucose in 30 minutes at 37°.

Histopathological Examination of Selected Tissue from cis-DDP Treated Animals

Male F-344 rats were treated with 0, 2.5, 5 or 7.5 mg/kg cis-DDP. Animals were sacrificed on day 4 and samples from the pancreas and kidney were fixed in buffered formalin (3.7% formaldehyde in 0.3 M sodium phosphate buffer, pH 7.2). After fixation, tissues were embedded in paraffin blocks, sectioned at 5 microns, mounted on glass slides and stained with hematoxylin and eosin under the supervision of Dr. Vance Sanger (Department of Pathology, Michigan State University, E. Lansing, MI). Histopathological examination of pancreatic tissue was performed by Dr. George Padgett (Department of Pathology, Michigan State University, E. Lansing, MI). Sections were evaluated for presence of intact islets, cellular necrosis, vacuolization and inflammatory cell infiltration. Histopathological examination of kidney tissue was performed by Dr. Keizo Maita (Department of Pathology, Center for Environmental Toxicology, Michigan State University, E. Lansing, MI). Renal tissue

was evaluated for coagulative necrosis, vacuolization, loss of brush border and nuclear enlargement.

Role of Adrenal Glands in cis-DDP Glucose Intolerance

Bilateral adrenalectomies (Adx) were performed in male F-344 rats anesthetized with sodium pentobarbital (50 mg/kg, i.p.) following retroperitoneal incisions. A control group of rats was subjected to sham operations in which the adrenal glands were inspected but not excised. All rats were allowed a 7 day recuperative period following surgery. During this period and for the duration of the experiment, adrenalectomized animals were allowed free access to saline (0.9%) in lieu of water to help maintain sodium balance. Following the recuperative period, rats were lightly anesthetized with ether and administered a single intravenous dose of 5 mg/kg cis-DDP or saline vehicle. The experimental groups included: Adx/control, Adx/cis-DDP and Sham/cis-DDP. Glucose tolerance was evaluated 2 and 4 days following drug or vehicle administration. Plasma from three identically treated animals was pooled and assayed for glucose as previously described. Plasma IRI and IRG was assayed in the fasting state and 15 minutes following a glucose load on day 2.

Role of the Kidney in cis-DDP Glucose Intolerance

Effect of Platinum on In Vivo Renal Function

Male F-344 rats, individually housed in stainless steel metabolism cages, were administered a single intravenous dose of 0, 2.5, 5, 7.5 or 15 mg/kg cis-DDP or trans-DDP or equimolar platinum doses of ammonium

tetrachloroplatinate (0, 3, 6, 9, or 18 mg/kg). A group of controls, pair-fed on a daily basis to each cis-DDP treatment group, was also studied. Twenty-four hour urine volume was determined following urine collection into glass flasks containing toluene to prevent microbial contamination and evaporation. Urine samples were collected prior to (day 0) and 1, 2, 4, 7 and 14 days following platinum treatment. Urine samples were also analyzed for: osmolality by a vapor pressure osmometer (Wescor, Inc., Logan, UT), sodium and potassium by flame photometry (IL Flame Photometer, Lexington, MA) and glucose by a spectrophotometric assay using a hexokinase reagent (Calbiochem, La Jolla, CA). Twenty-four hour urinary osmolar, sodium, potassium and glucose excretion was calculated from the observed volume and measured concentrations.

At the end of days 0, 1, 2, 4, 7, and 14, animals were lightly anesthetized with ether and approximately 1 ml blood was sampled from the orbital sinus cavity. Blood was allowed to clot, centrifuged for 10 minutes at 2000 x g and the serum fraction separated and analyzed for urea nitrogen using a urease-Berthelot reagent (Sigma Chemical Co., St. Louis, MO).

Effect of Platinum on Renal Organic Ion Transport

Four days following cis-DDP administration (0, 1.25, 2.5 or 5 mg/kg), animals were weighed and killed by decapitation. Kidneys were quickly removed, decapsulated, weighed and placed in cold isotonic saline (0.9% NaCl). The ability of renal cortical slices to actively accumulate an organic anion, p-aminohippurate (PAH), and an organic cation, tetraethylammonium (TEA), was determined. Renal slices were prepared

freehand and approximately 100 mg tissue was incubated in phosphate buffered medium (Cross and Taggart, 1950) containing 7.4×10^{-5}M [^{14}C]PAH (0.02 mCi/ml) (New England Nuclear, Boston, MA) and 1×10^{-3}M [^{14}C]TEA (0.02 mCi/ml) (New England Nuclear, Boston, MA). Incubations were performed in a Dubnoff metabolic shaker at 25°C for 90 minutes under 100% oxygen. After incubation, slices were quickly removed, blotted, weighed and prepared for analysis of PAH and TEA concentration of both slice and media preparations by liquid scintillation spectroscopy. Data are expressed as slice (dpm/g tissue) to medium (dpm/ml media) or S/M concentration ratios.

To determine the *in vitro* effect of cis-DDP on renal organic ion transport, renal cortical slices were prepared from untreated adult male F-344 rats as described above. Slices were incubated in phosphate-buffered medium (Cross and Taggart, 1950) containing 7.4×10^{-5}M PAH, 1×10^{-3}M [^{14}C]TEA (0.02 mCi/ml), and 0, 300, 400, 500 or 600 µg/ml cis-DDP. Following incubation procedures as described above, tissue and media were prepared for the spectrophotometric determination of PAH according to Smith *et al.* (1945). TEA concentrations of tissue and media was determined by liquid scintillation spectroscopy. Data are expressed as S/M concentration ratios.

Effect of cis-DDP on Renal Clearance of Inulin and PAH

The effects of cis-DDP on the renal clearance of inulin (C_{inulin}) and PAH (C_{PAH}) were determined *in vivo* four days following administration of 5 mg/kg cis-DDP or a saline vehicle. A pair-fed control group was also studied. Rats were anesthetized with 50 mg/kg sodium pentobarbital, intraperitoneally. Body temperature was maintained at 37°

using heat lamps. A PE50 cannula was inserted into the bladder and urine was collected into preweighed vials. The femoral vein was cannulated for infusion of a saline solution containing $2x10^{-3}$M [^{3}H]inulin (0.5 μCi/ml) and $3x10^{-2}$M [^{14}C]PAH (0.5 μCi/ml) infused at 0.018 ml/minutes using a Harvard infusion pump. The duration of the clearance experiment approximated 3 hours and included: an equilibration period of 90 minutes from the beginning of infusion to the initiation of urine collections, two 20-minute urine collections (prevolume expansion period), one 5-minute volume expansion period followed by an equilibration period of 10 minutes and a final 20-minute urine collection (post-volume expansion period). Blood (300 μl) was sampled from the femoral artery at the midpoint of each urine collection. Volume expansion was accomplished by infusing a 1:4 rat plasma:saline solution at 4% body weight. [^{14}C]PAH and [^{3}H]inulin in plasma and urine was simultaneously determined by dual-label counting techniques using a Packard Tri-Carb liquid scintillation counter.

Effect of Mannitol Pretreatment on cis-DDP Glucose Intolerance

Pretreatment of animals with mannitol was conducted according to the method described by Pera et al. (1979). Rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and the right jugular vein cannulated with PE50 tubing. A 10% mannitol solution (in 0.45% NaCl) was infused at a dose of 2.4 g/kg over a 30 minute period using a Harvard infusion pump. After 25 minutes, a bolus injection of cis-DDP (5 mg/kg) or saline vehicle was infused via the intravenous tubing. Mannitol was infused for the remaining few minutes. Control animals also received

mannitol and were pair-fed to the mannitol/cis-DDP group. In addition, another group of animals was subjected to similar surgical procedures with the exception that they received cis-DDP alone, without mannitol pretreatment (Sham/cis-DDP). Thus, there were three experimental groups: Mannitol/Control, Mannitol/cis-DDP and Sham/cis-DDP. On day 4, glucose tolerance was evaluated as previously described. Plasma obtained from three identically treated rats was pooled and assayed for glucose, IRI and IRG. Following completion of glucose tolerance tests, rats were anesthetized with pentobarbital (50 mg/kg, i.p.) and blood sampled from the abdominal aorta. Plasma was assayed for urea nitrogen as previously described.

Effect of Selected Nephrotoxicants on Glucose Tolerance

In addition to cis-DDP (5 mg/kg), three agents known to impair renal function were studied for their effects on glucose tolerance: cephaloridine (1000 mg/kg, i.p.) (Eli Lilly and Co., Indianapolis, IN), gentamicin (30 mg/kg, i.p. x 2x/day x 8 days) (Schering Pharmaceutical Corp., Kenilworth, NJ) and glycerol (10 ml/kg, i.m., 50% w/v solution). Controls received a saline vehicle. Glucose tolerance was evaluated on those days in which renal functional impairment was manifested as an elevation in blood urea nitrogen as determined by preliminary experiments. Thus, glucose tolerance was evaluated on:

Day 1 -- glycerol

Day 4 -- cis-DDP and cephaloridine

Day 9 -- gentamicin

Injections were staggered in such a way that evaluation of glucose tolerance for all treatments was conducted on the same day. Serial plasma samples were assayed for glucose as previously described. Following the two hr glucose tolerance test, animals were anesthetized with pentobarbital (50 mg/kg, i.p.) and blood sampled from the abdominal aorta. Serum samples were measured for urea nitrogen and creatinine concentrations (Sigma Chemical Co., St. Louis, MO). Following exsanguination, kidneys were removed and wet weight determined.

Biochemical Correlates of cis-DDP Glucose Intolerance

Effects of cis-DDP on Serum Sodium, Potassium, Calcium and Phosphorus

Male F-344 rats were treated with a single intravenous dose of 0, 2.5, 5 or 7.5 mg/kg cis-DDP. Controls pair-fed to the 7.5 mg/kg group were also studied. On day 4, serum was obtained from abdominal aortic blood of anesthetized animals and assayed for sodium and potassium by flame photometry as previously described. Serum calcium was quantified using a Varian AA-375 atomic absorption flame photometer. For calcium determinations, potassium (5000 µg/ml) was added to serum samples and calcium measured at 422.7 nm using a nitrous oxide acetylene flame. Serum phosphorus was measured using a modification of the method described by Fiske and Subbarow (1925).

Gluconeogenic Enzyme Activity

Hepatic and renal glucose-6-phosphatase (G-6-Pase) and fructose 1,6-diphosphatase (FDPase) activities were determined in male F-344 rats four days following a single intravenous administration of 0, 2.5, 5 or

7.5 mg/kg cis-DDP. A group of controls pair-fed to the 7.5 mg/kg group was also studied. All animals were fasted overnight (16 hours) prior to the day of experimentation. On day 4, animals were sacrificed by decapitation, blood collected in chilled test tubes for glucose determination and liver and kidneys were rapidly removed and placed in ice-cold buffers.

For measurement of G-6-Pase activity, 250 mg tissue was weighed, minced, rinsed and homogenized in 9.75 ml of ice-cold 0.1 M citrate buffer, pH 6.5, using 4 passes of a motor driven Potter-Elvehjem homogenizer. The homogenates were then filtered through cheesecloth and used for the *in vitro* determination of G-6-Pase activity according to the method of Harper (1965). Following five minutes of preincubation of homogenates (0.1 ml) in a Dubnoff metabolic shaker at 37°, either 0.1 ml of 0.08 M glucose-6-phosphate (substrate) or citrate buffer (tissue blank) was added to samples. After exactly 15 minutes, 2 ml of 10% TCA was added to the reaction mixture and samples placed on ice. Following centrifugation of samples (2000 x g for 10 minutes), product formation was estimated in the resulting supernatant fraction by the spectrophotometric determination of phosphate according to Fiske and Subbarow (1925). Protein concentration of whole homogenates was determined according to Lowry *et al.* (1951) and results expressed as μmoles phosphate/hour/mg protein.

FDPase activity was quantified in hepatic and renal tissue by preparing 5% homogenates in ice-cold 0.15 M KCl buffer, pH 7.4 using four passes of a motor driven homogenizer. The homogenates were centrifuged

at 100,000 x g at 0° for 30 minutes using a Beckman L8-55 ultracentrifuge. The supernatant fraction was then assayed for FDPase activity by a modification of the method of Pogell and McGilvery (1952). Incubation media contained 0.05 M sodium borate buffer, pH 9.5, 0.1 ml of 0.05 M $MgSO_4$, 0.1 ml of 0.05 M fructose 1,6-diphosphate and supernatant fluid (kidney, 0.3 ml; liver, 0.2 ml), yielding a total reaction volume of 1.0 ml. Following preincubation of samples containing buffer, $MgSO_4$ and supernatant for 5 minutes at 37°, substrate was added and samples were incubated for exactly 15 minutes. The enzymatic reaction was stopped by the addition of 1 ml of 10% TCA. Following centrifugation (2000 x g for 10 minutes), samples were measured for product formation by the spectrophotometric determination of phosphate (Fiske and Subbarow, 1925). Protein was determined in the 100,000 x g supernatant according to the method of Lowry et al. (1951) and results expressed as μmoles phosphate/hr/mg protein. Both enzyme assays were carried out under strictly linear conditions with respect to time and protein concentration.

Endocrine Correlates of cis-DDP Glucose Intolerance

Plasma and Pancreatic IRI and IRG Concentrations

Blood from treated animals in above experiment was collected in chilled test tubes containing EDTA and Traysylol for determinations of plasma IRI and IRG. In addition, pancreatic tissue from these animals was rapidly excised, weighed and homogenized for 10 seconds in an ice-cold 2.1% sulfuric acid/80% ethanol mixture. Pancreatic tissue from the

duodenal lobe (head) and the remaining tissue adjacent to the spleen and stomach (body and tail) were individually analyzed for IRI and IRG concentrations. Extractions were carried out under ice-cold conditions using a ratio of 100 mg tissue:1 ml acid/ethanol solution. Samples were incubated overnight at 4°. The following day samples were centrifuged at 2000 x g for 10 minutes at 4°C. Supernatants were appropriately diluted with 0.05 M phosphate buffer, pH 7.6, containing 0.025 M EDTA, 0.9% NaCl, 1% bovine serum albumin and 10% Traysylol. Samples were frozen at 0° and later assayed for IRI and IRG as previously described.

Components of Plasma IRG

Four days following treatment with 0, 2.5, 5 or 7.5 mg/kg cis-DDP, animals were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and blood sampled from the abdominal aorta twenty minutes following administration of the anesthetic. Blood was collected in chilled test tubes containing EDTA and Traysylol. Plasma was frozen at 0° and later analyzed for IRG as previously described ("total" plasma IRG). In addition, plasma reactivity to Unger's 30K antibody, which utilizes an anti-porcine/bovine glucagon obtained from rabbits, was also measured and designated as "true pancreatic" plasma IRG. "Extra-pancreatic" plasma IRG was calculated as the difference between "total" and "true pancreatic" plasma IRG.

Both "total" and "true pancreatic" IRG were also measured in plasma of animals from experiments evaluating the effect of mannitol pretreatment and the effect of selected nephrotoxicants.

Glucagon Resistance

Four days following drug treatment (0, 2.5, 5 or 7.5 mg/kg cis-DDP), rats were fasted 4-5 hours and were evaluated for glucagon resistance by measuring the glycemic response to an intraperitoneal administration of porcine glucagon (1 mg/kg) (Sigma Chemical Co., St. Louis, MO). Blood was sampled by orbital sinus puncture in unanesthetized animals before and 10, 20, 30 and 60 minutes following glucagon administration. Plasma was assayed for glucose as previously described.

Insulin Resistance

Four days following administration of 5 mg/kg cis-DDP or a saline vehicle, rats were fasted 4-5 hours and then evaluated for insulin resistance by measuring the glycemic response to an intraperitoneal administration of porcine insulin (0.28 IU/kg) (Sigma Chemical Co., St. Louis, MO). Blood was sampled from the orbital sinus cavity in unanesthetized animals before and 10, 15, 20, 30 and 60 minutes following insulin administration. Plasma was assayed for glucose as previously described.

Statistical Analyses

All data are expressed as means ± SEM. All data were analyzed by analysis of variance and treatment means were compared using the Student-Newman-Keuls or the Least-Significant-Difference tests (Sokal and Rohlf, 1969). The criterion of significance was $p<0.05$.

RESULTS

Food Intake

A dose-related depression in food intake was observed following treatment with cis-DDP (Figure 4). Daily food consumption averaged 16, 12, 11, 9 and 8 grams one day following treatment with 0, 2.5, 5, 7.5 or 15 mg/kg cis-DDP, respectively (Figure 4). Animals treated with 7.5 or 15 mg/kg cis-DDP did not survive the duration of the 14 day experiment. In animals treated with 5 mg/kg cis-DDP, food intake was depressed through day 4, increased modestly on day 7, and was comparable to controls on day 14 (Figure 4). In contrast, administration of equimolar doses of trans-DDP did not significantly alter daily food intake at any time examined (Table 1). Animals treated with 18 mg/kg tetrachloroplatinate consumed significantly less food than controls one day following treatment; however, food consumption in this treatment group was not different from controls on days 2, 4, 7 and 14 (Table 1). Smaller doses of tetrachloroplatinate did not significantly affect daily food intake (Table 1).

Body Weight

Significant weight loss was apparent 2 and 4 days following treatment with 5, 7.5 or 15 mg/kg cis-DDP (Figure 5). Treatment with 5 mg/kg cis-DDP also resulted in a significant reduction in body weight on day

Figure 4. Effect of cis-DDP on daily food intake. Each point with vertical line represents the mean ± SEM of four to five determinations. Animals treated with 7.5 or 15 mg/kg cis-DDP did not survive the duration of the 14 day experiment. Asterisks indicate a significant difference from controls on the corresponding day ($p<0.05$).

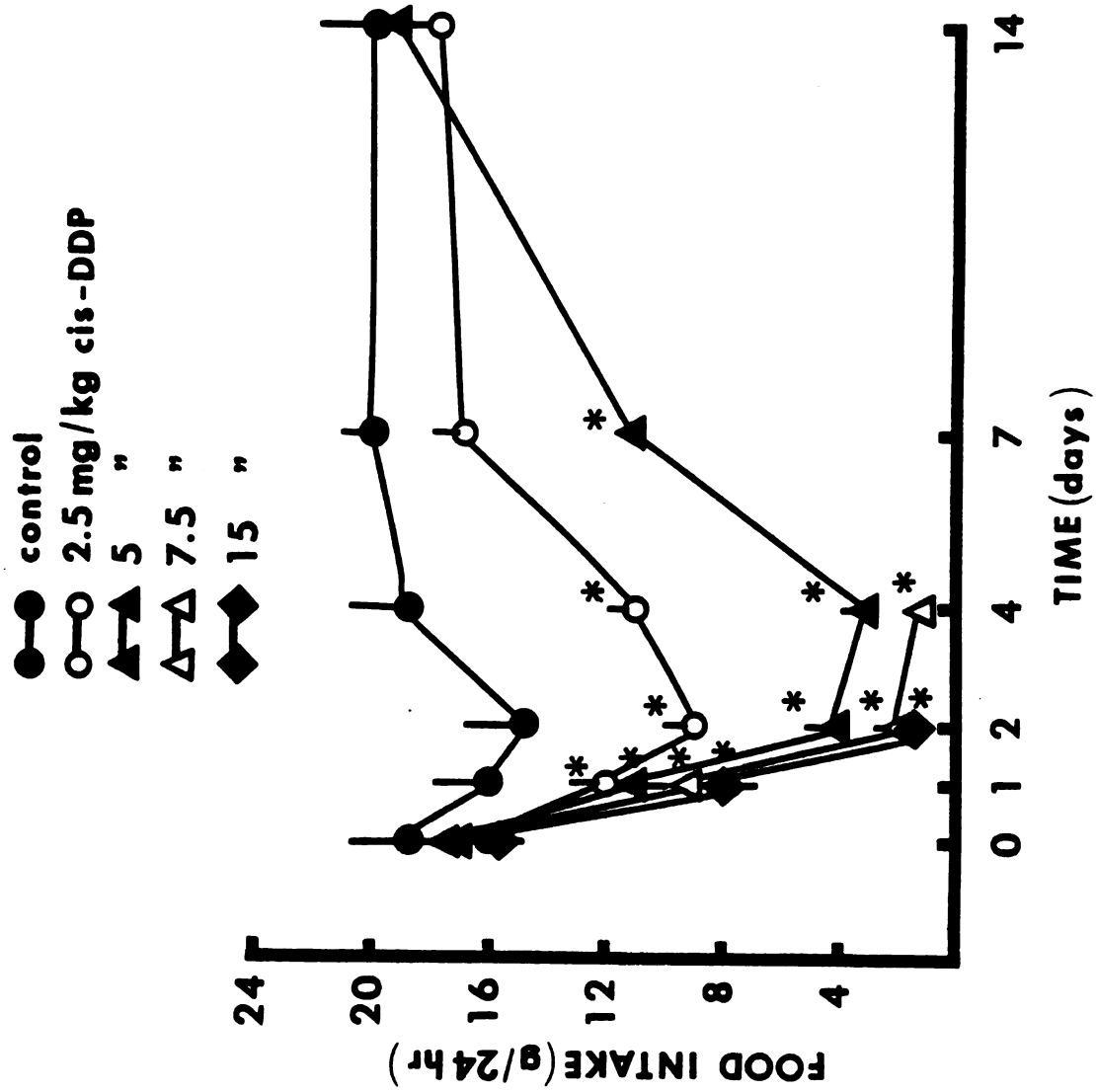
control
2.5 mg/kg cis-DDP
5 "
7.5 "
15 "
FOOD INTAKE (g/24hr)
24
20
16
12
8
4
0 1 2 4 7 14
TIME (days)

Figure 4

Figure 4. Effect of cis-DDP on daily food intake. Each point with vertical line represents the mean ± SEM of four to five determinations. Animals treated with 7.5 or 15 mg/kg cis-DDP did not survive the duration of the 14 day experiment. Asterisks indicate a significant difference from controls on the corresponding day ($p<0.05$).

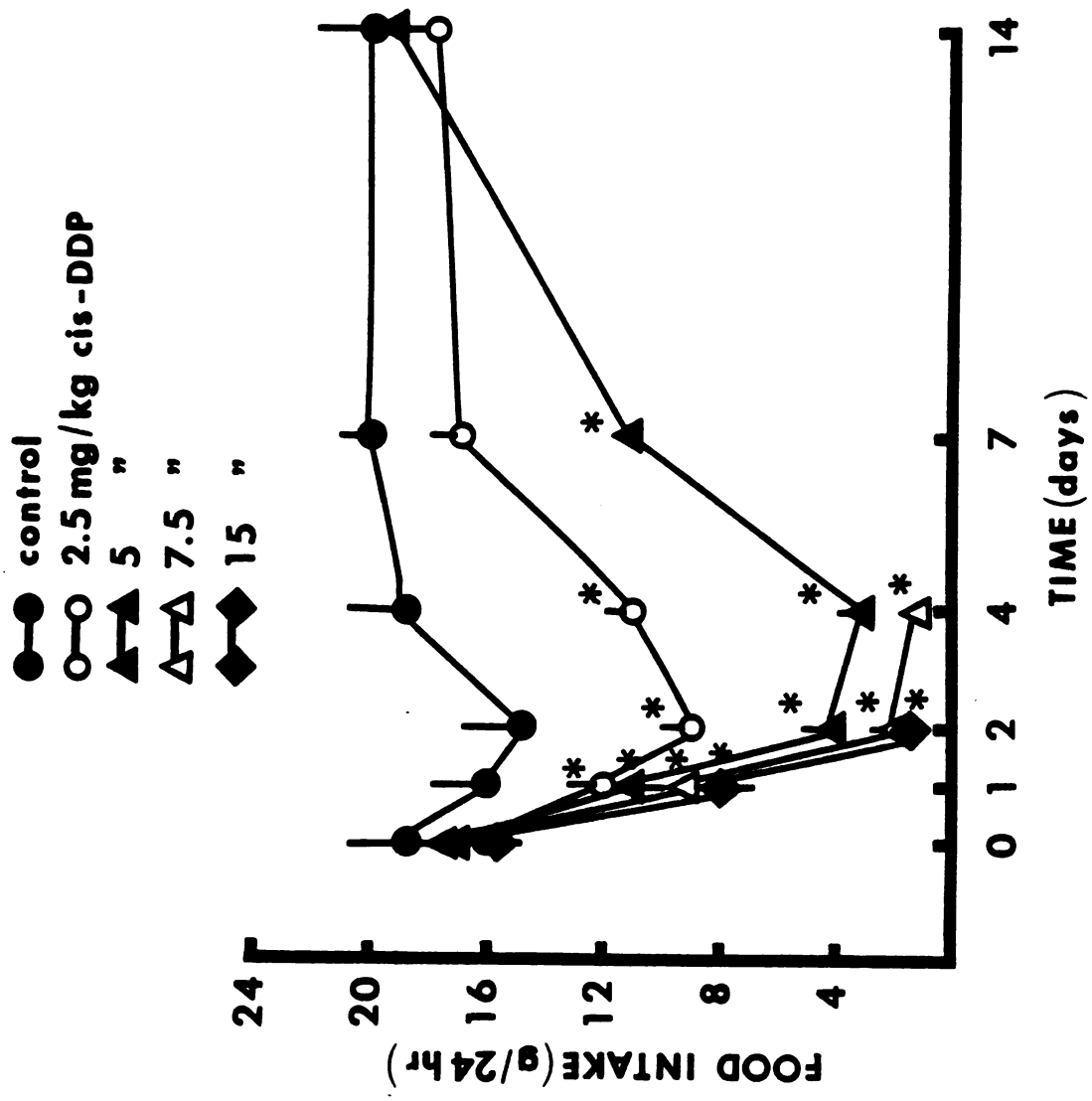
control
2.5 mg/kg cis-DDP
5 "
7.5 "
15 "
FOOD INTAKE (g/24hr)
24
20
16
12
8
4
0 1 2 4 7 14
TIME (days)

Figure 4

TABLE 1

Effects of Platinum Treatment on Daily Food Intake
(g food/24 hours)

	Time (days after administration)					
	0	1	2	4	7	14
Trans-DDP (mg/kg)						
0	17±2[a]	11±2	13±1	14±1	15±1	12±1
2.5	15±1	11±2	12±1	13±1	16±1	18±2
5	16±2	13±2	13±2	16±1	16±1	16±1
7.5	16±2	11±1	12±1	14±1	14±1	14±2
15	14±1	11±2	9±1	13±1	15±1	16±1
$(NH_4)_2PtCl_4$ (mg/kg)						
0	15±1	12±2	15±1	15±1	15±2	18±3
3	13±1	12±1	14±2	15±1	15±1	19±1
6	15±1	9±1	11±1	13±2	15±1	20±1
9	17±1	11±2	14±1	15±2	15±1	16±2
18	14±1	7±2[b]	10±1	13±2	12±2	18±2

[a]Values are expressed as means ± SEM of four to five determinations.

[b]Significantly different from controls on corresponding day ($p<0.05$).

Figure 5. Top: Effect of cis-DDP on body weight. Data are expressed as percent of initial or pretreatment (day 0) body weight. Each point with vertical line represents the mean ± SEM of four to five determinations. Animals treated with 7.5 or 15 mg/kg cis-DDP did not survive the duration of the 14 day experiment. Asterisks indicate a significant difference from controls on the corresponding day ($p<0.05$).

Bottom: Effect of reduced food intake (pair-feeding) on body weight. Animals were offered the measured amount of food consumed by their drug treated partners. Each symbol indicates the drug treatment group to which animals were pair-fed. Asterisks indicate a significant difference from controls on the corresponding day ($p<0.05$).

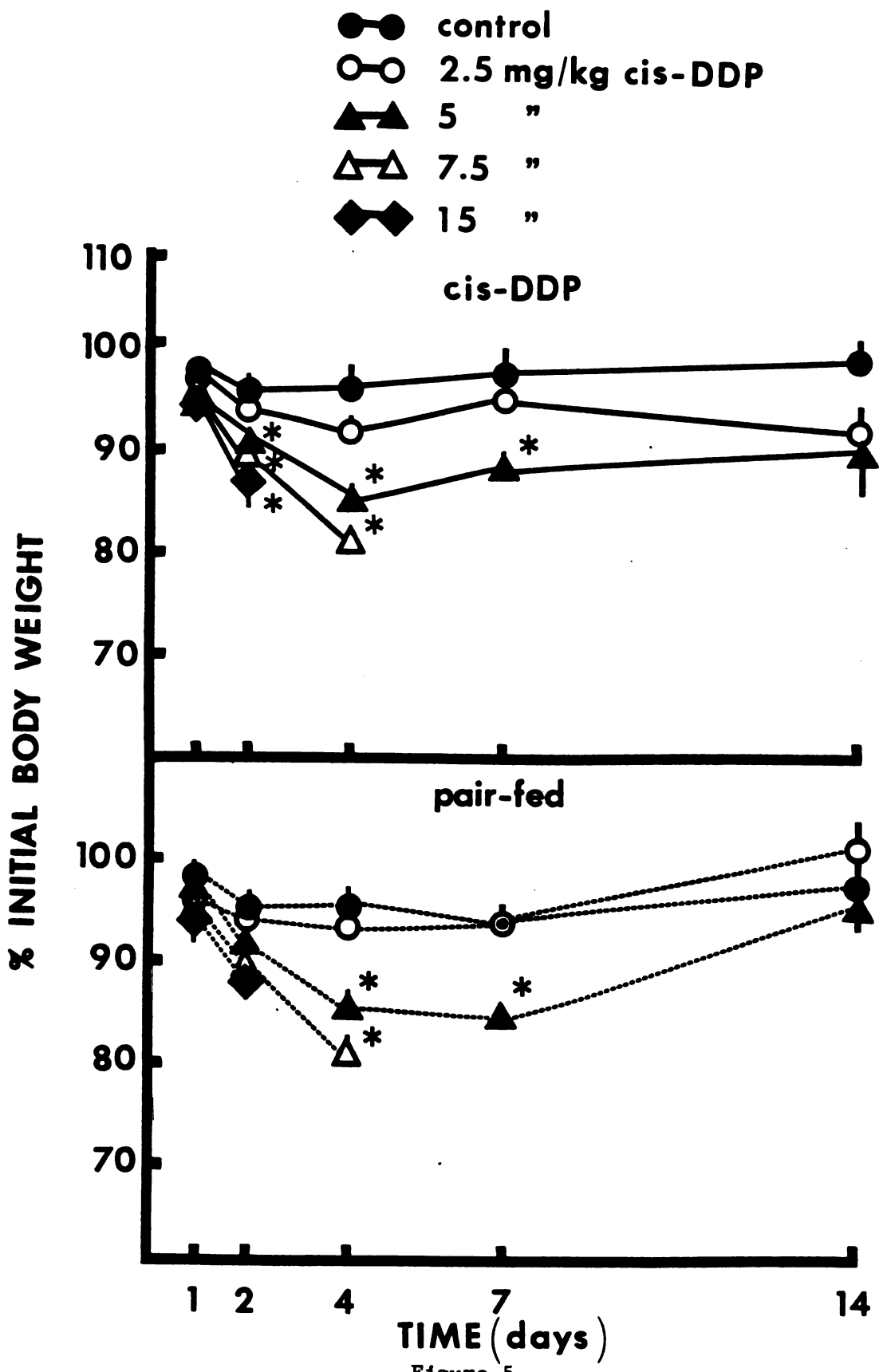
control
2.5 mg/kg cis-DDP
5 "
7.5 "
15 "
cis-DDP
pair-fed
% INITIAL BODY WEIGHT
110
100
90
80
70
1
2
4
7
14
TIME (days)

Figure 5

7; however, by day 14, body weights of these treated animals were comparable to those of ad-libitum fed controls (Figure 5). No effect on body weight was evident following treatment with 2.5 mg/kg cis-DDP (Figure 5). Animals pair-fed to cis-DDP treated groups (5 or 7.5 mg/kg) similarly lost a significant amount of weight on days 4 and 7 (Figure 5). Body weights of pair-fed partners to the 5 mg/kg group were comparable to controls on days 1, 2 and 14 (Figure 5). In contrast, neither trans-DDP nor tetrachloroplatinate significantly affected body weight at any time examined (Table 2).

Effect of Platinum on Serum Glucose in Nonfasting Animals

Hyperglycemia was evident one day following treatment with 7.5 or 15 mg/kg cis-DDP (210±15 and 210±3 mg/dl, respectively) (Figure 6). Hyperglycemia persisted in these animals through day 2, reaching a concentration of 541±89 mg/dl in the latter treatment group (Figure 6). Treatment with 5 mg/kg cis-DDP resulted in a significant elevation in serum glucose only on day 2 (Figure 6). In contrast, administration of equimolar doses of trans-DDP or ammonium tetrachloroplatinate did not significantly alter serum glucose concentration at any time examined (Figure 6).

Effect of cis-DDP on Serum Glucose in Fasting Animals

Animals treated with 7.5 or 10 mg/kg cis-DDP exhibited fasting hyperglycemia 0.5 days following drug treatment (Figure 7). In both of these treatment groups, hyperglycemia was apparent immediately prior to death, reaching concentrations of 527±101 mg/dl 1.5 days following

TABLE 2

Effects of Platinum Treatment on Percent of Initial Body Weight

	Time (days after administration)				
	1	2	4	7	14
Trans-DDP (mg/kg)					
0	99±1[a]	97±1	97±2	97±2	100±3
2.5	99±1	96±1	97±1	100±2	105±1
5	98±1	96±1	96±1	97±2	102±2
7.5	98±1	95±1	96±1	98±2	101±2
15	97±1	95±1	94±1	96±2	98±1
$(NH_4)_2PtCl_4$ (mg/kg)					
0	99±1	97±2	95±2	97±2	102±2
3	97±1	97±1	95±1	96±2	100±3
6	98±1	95±2	96±2	97±1	101±1
9	98±1	96±1	96±1	96±2	99±2
18	95±1	93±2	91±2	93±3	99±1

[a]Values are expressed as means ± SEM of four to five determinations. Values represent percent of body weight on day 0.

Figure 6. Effect of cis-DDP (top), trans-DDP (center) or ammonium tetrachloroplatinate (bottom) on serum glucose concentration in non-fasting animals. Each point with vertical line represents the mean ± SEM of four to five determinations. Animals treated with 7.5 or 15 mg/kg cis-DDP did not survive the duration of the 14 day experiment. The stippled area represents the mean ± 95% confidence interval of pair-fed animals. Asterisks indicate a significant difference from both ad-libitum and pair-fed controls ($p<0.05$).

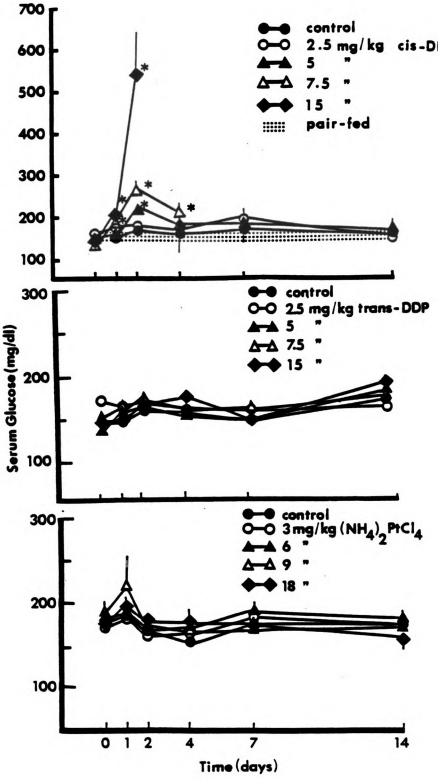
control
2.5 mg/kg cis-DDP
5 "
7.5 "
15 "
pair-fed
control
2.5 mg/kg trans-DDP
5 "
7.5 "
15 "
control
3 mg/kg $(NH_4)_2PtCl_4$
6 "
9 "
18 "
Serum Glucose (mg/dl)
Time (days)
0 1 2 4 7 14
100 200 300 400 500 600 700

Figure 6

Figure 7. Effects of cis-DDP on serum glucose concentration in fasting animals. Each point with vertical line represents the mean ± SEM of four determinations. Animals treated with 7.5 or 10 mg/kg cis-DDP did not survive the duration of the 7 day experiment. Asterisks indicate a significant difference from controls on the corresponding day ($p<0.05$).

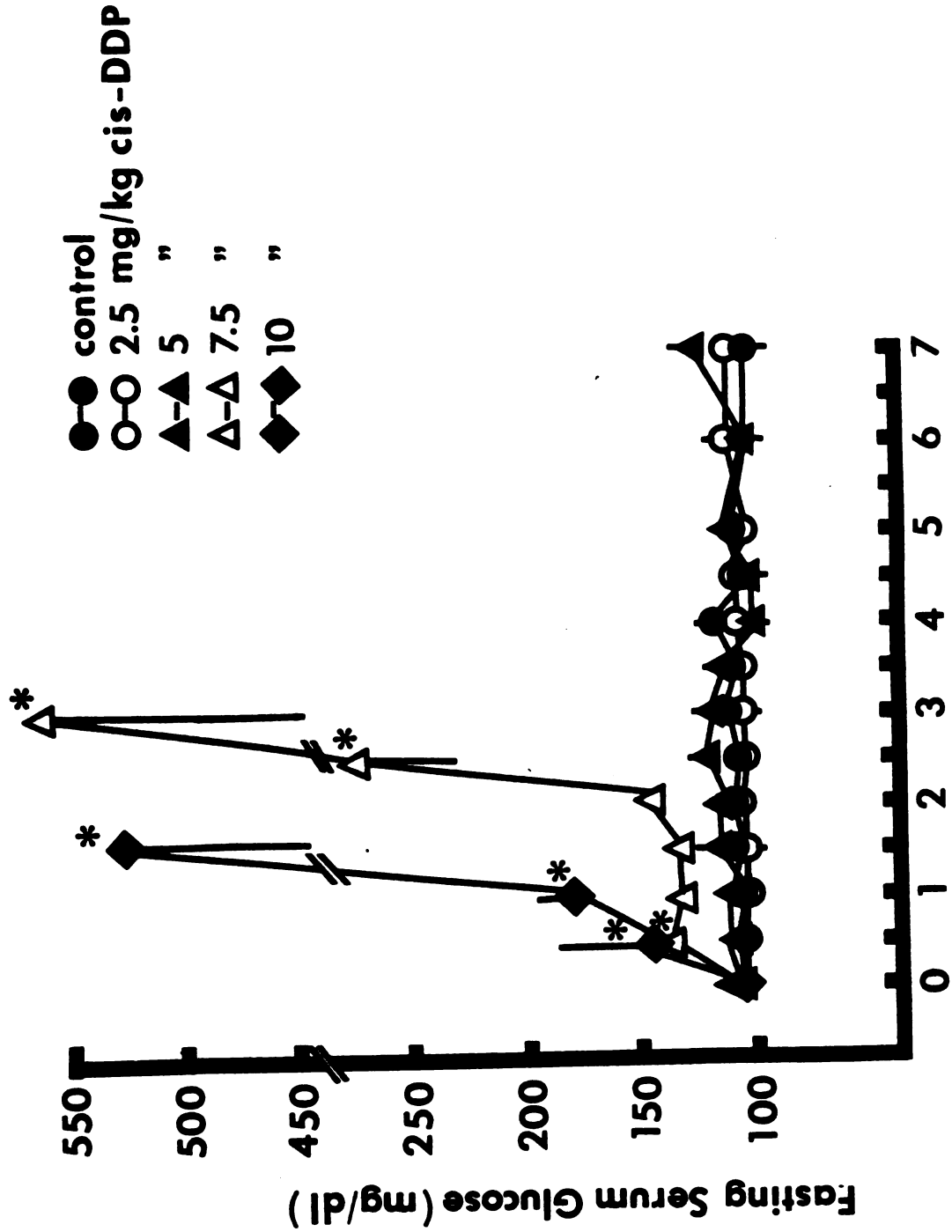
control
2.5 mg/kg cis-DDP
5 "
7.5 "
10 "
Fasting Serum Glucose (mg/dl)
550
500
450
250
200
150
100
0 1 2 3 4 5 6 7
Time (days)

Figure 7

administration of 10 mg/kg cis-DDP and 560±136 mg/dl 3 days following treatment with 7.5 mg/kg cis-DDP (Figure 7). Smaller doses of cis-DDP (2.5 or 5 mg/kg) did not affect fasting serum glucose concentration at any time examined (Figure 7).

Effect of cis-DDP on Glucose Tolerance

Fasting plasma glucose concentration was two times greater in animals treated with 7.5 mg/kg cis-DDP compared to both ad-libitum and pair-fed controls (Figure 8). These differences, however, were not statistically significant. Although the glucose tolerance curve of the 2.5 mg/kg group was comparable to controls, plasma glucose concentrations of animals treated with 7.5 mg/kg cis-DDP were significantly elevated throughout the two hour glucose tolerance test. At 120 minutes following a glucose load, plasma glucose concentration was six times greater in this treatment group compared to controls (Figure 8).

Fasting plasma IRI concentrations of cis-DDP treated animals were not significantly different than ad-libitum fed controls; however, those of the 7.5 mg/kg group were four times greater than their pair-fed partners (Figure 9). Fifteen minutes following a glucose challenge, plasma IRI concentrations of ad-libitum and pair-fed controls increased approximately 490 and 1100%, respectively, from fasting concentrations. Animals treated with 2.5 mg/kg cis-DDP exhibited a similar plasma IRI response to glucose stimulation as controls. However, plasma IRI did not change from fasting concentrations upon glucose administration in the 7.5 mg/kg group (Figure 9). The lack of insulin response to a glucose stimulus in these cis-DDP treated animals persisted throughout the two hour experimental period (Figure 9).

Figure 8. Effect of cis-DDP on plasma glucose concentration in the fasting state (0) and 15, 30, 60, and 120 minutes following a glucose load (2 g/kg, i.p.). Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Pair-fed animals were offered the measured amount of food consumed by the 7.5 mg/kg group. Asterisks indicate a significant difference from both ad-libitum and pair-fed controls at the corresponding time point ($p<0.05$).

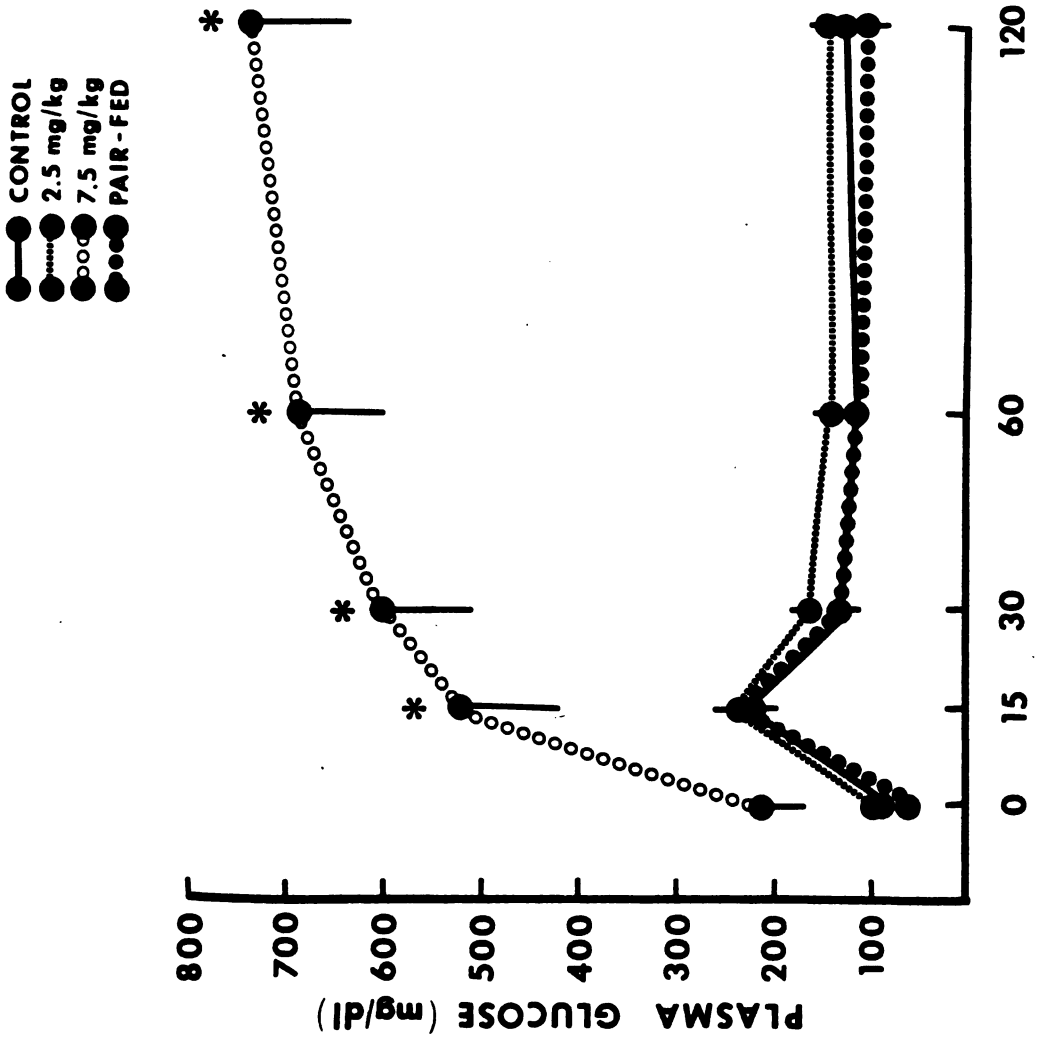
CONTROL
2.5 mg/kg
7.5 mg/kg
PAIR-FED
PLASMA GLUCOSE (mg/dl)
800
700
600
500
400
300
200
100
0
15
30
60
120
TIME (min)

Figure 8

Figure 9. Effect of cis-DDP on plasma immunoreactive (IRI) in the fasting state (0) and 15, 30, 60, and 120 minutes following a glucose load (2 g/kg, i.p.). Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Pair-fed animals were offered the measured amount of food consumed by 7.5 mg/kg group. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, at the corresponding time point ($p<0.05$).

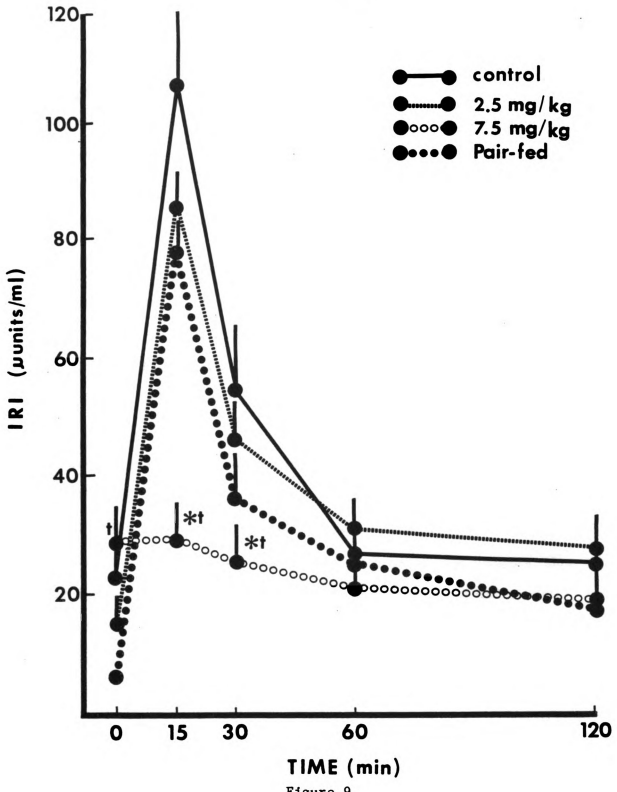
120
100
80
60
40
20
IRI (μunits/ml)
control
2.5 mg/kg
7.5 mg/kg
Pair-fed
t
*t
*t
0
15
30
60
120
TIME (min)

Figure 9

Fasting plasma IRG of the 7.5 mg/kg group averaged 777±155 pg/ml whereas ad-libitum and pair-fed controls averaged 254±17 and 178±3 pg/ml, respectively (Figure 10). Similarly, plasma IRG concentrations in the 7.5 mg/kg group were significantly elevated throughout the two hour glucose tolerance test (Figure 10). Administration of 2.5 mg/kg cis-DDP did not significantly affect plasma IRG concentrations in either the fasting or glucose stimulated state (Figure 10).

Effect of Platinum on Glucose Tolerance: Time Course and Dose Response

Fasting plasma glucose concentrations were not significantly elevated 2, 4, 7 or 14 days following administration of cis-DDP (Figures 11-14), trans-DDP (Figures 11-14) or ammonium tetrachloroplatinate (Figures 15 and 16). However, on day 2, plasma glucose was markedly elevated 15, 30, 60 and 120 minutes following a glucose load in the 5 mg/kg cis-DDP group compared to both ad-libitum and pair-fed controls (Figure 11). In contrast, equimolar or greater than equimolar doses of trans-DDP did not result in plasma glucose concentrations different than controls during the two hour glucose tolerance test (Figure 11).

On day 4, animals treated with 5 mg/kg cis-DDP, but not those treated with equimolar or greater than equimolar doses of its trans isomer, exhibited profound hyperglycemia following a glucose load, reaching a concentration of 379±10 mg/dl at 15 minutes compared to ad-libitum and pair-fed control values of 232±20 and 300±13 mg/dl, respectively (Figure 12). Hyperglycemia in the 5 mg/kg cis-DDP group was evident following a glucose load at every time point examined (Figure 12). Plasma glucose was significantly elevated only at 15 minutes following glucose administration in the pair-fed group (Figure 12).

Figure 10. Effect of cis-DDP on plasma immunoreactive glucagon (IRG) in the fasting state (0) and 15, 30, 60, and 120 minutes following a glucose load (2 g/kg, i.p.). Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Pair-fed animals were offered the measured amount of food consumed by 7.5 mg/kg group. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, at the corresponding time point ($p<0.05$).

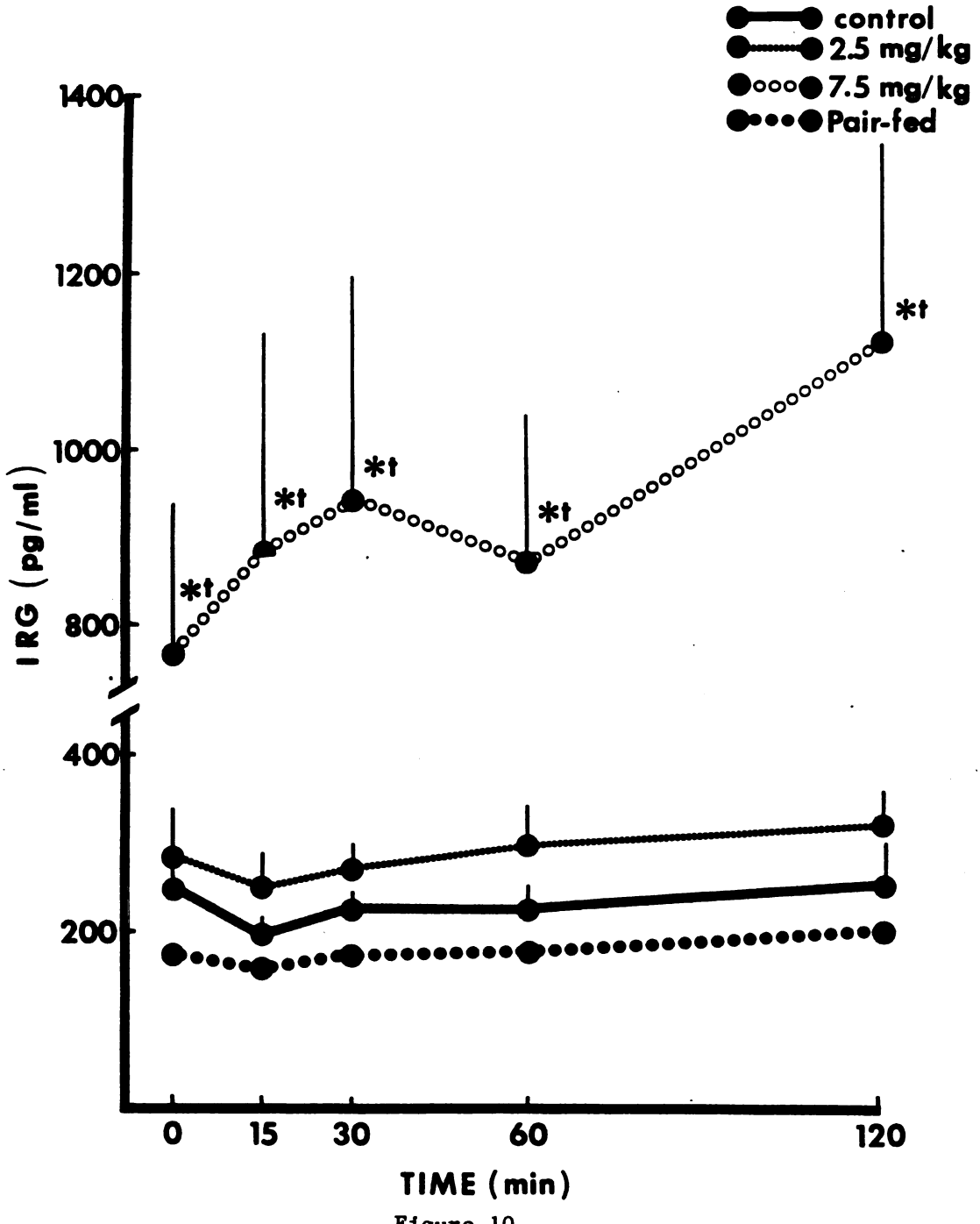
control
2.5 mg/kg
7.5 mg/kg
Pair-fed
1400
1200
1000
800
400
200
IRG (pg/ml)
*t
*t
*t
*t
*t
0
15
30
60
120
TIME (min)

Figure 10

Figure 11. Effect of cis-DDP (left) and trans-DDP (right) on glucose tolerance two days following DDP administration. Plasma glucose concentration is plotted against time following a glucose load (2 g/kg, i.p.). Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, at the corresponding time point ($p<0.05$).

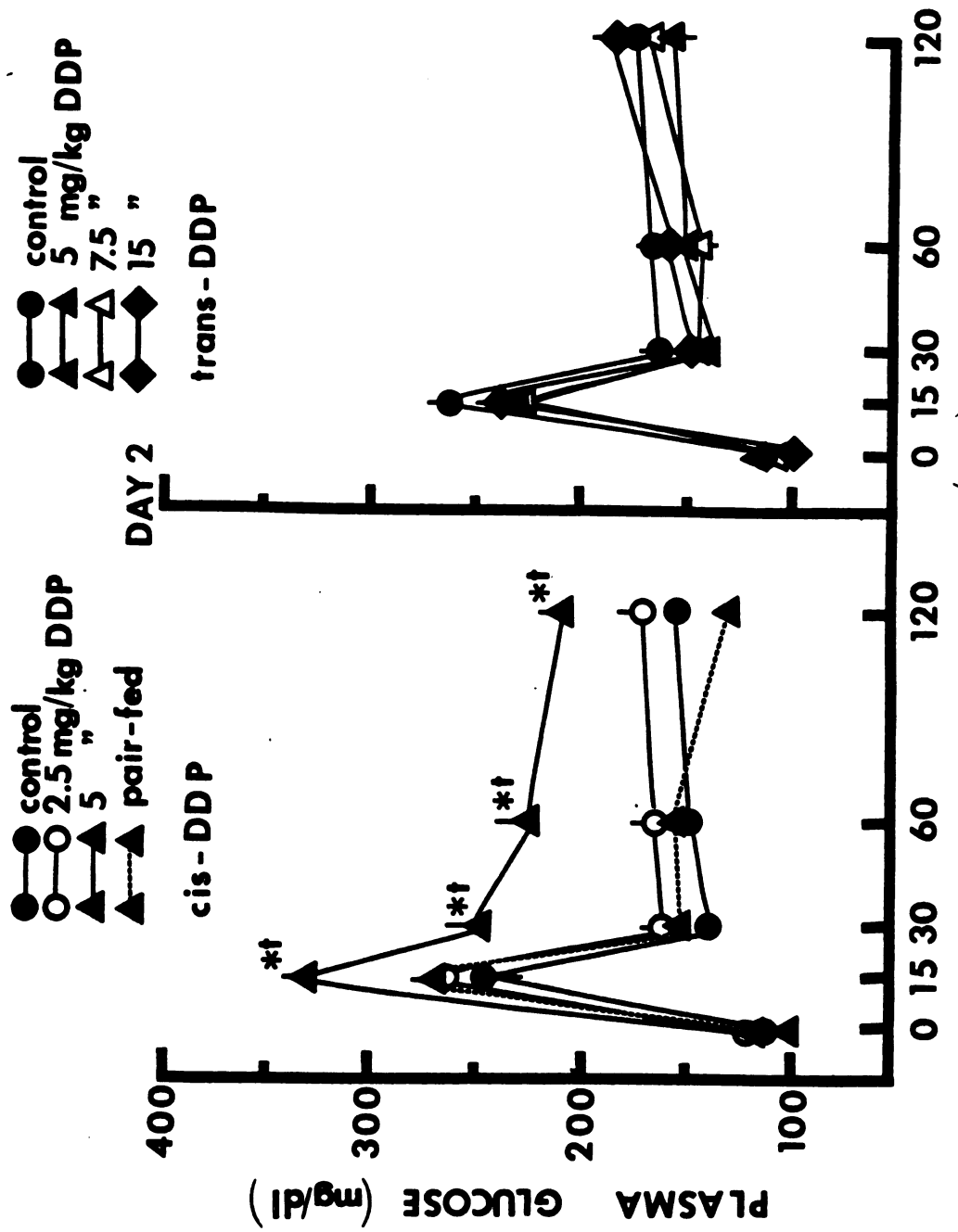
control
2.5 mg/kg DDP
5 "
pair-fed
cis-DDP
control
5 mg/kg DDP
7.5 "
15 "
trans-DDP
DAY 2
*†
PLASMA GLUCOSE (mg/dl)
400
300
200
100
0 15 30 60 120
TIME (min)
0 15 30 60 120

Figure 11

Figure 12. Effect of cis-DDP (left) and trans-DDP (right) on glucose tolerance four days following DDP administration. Plasma glucose concentration is plotted against time following a glucose load (2 g/kg, i.p.). Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, at the corresponding time point ($p<0.05$).

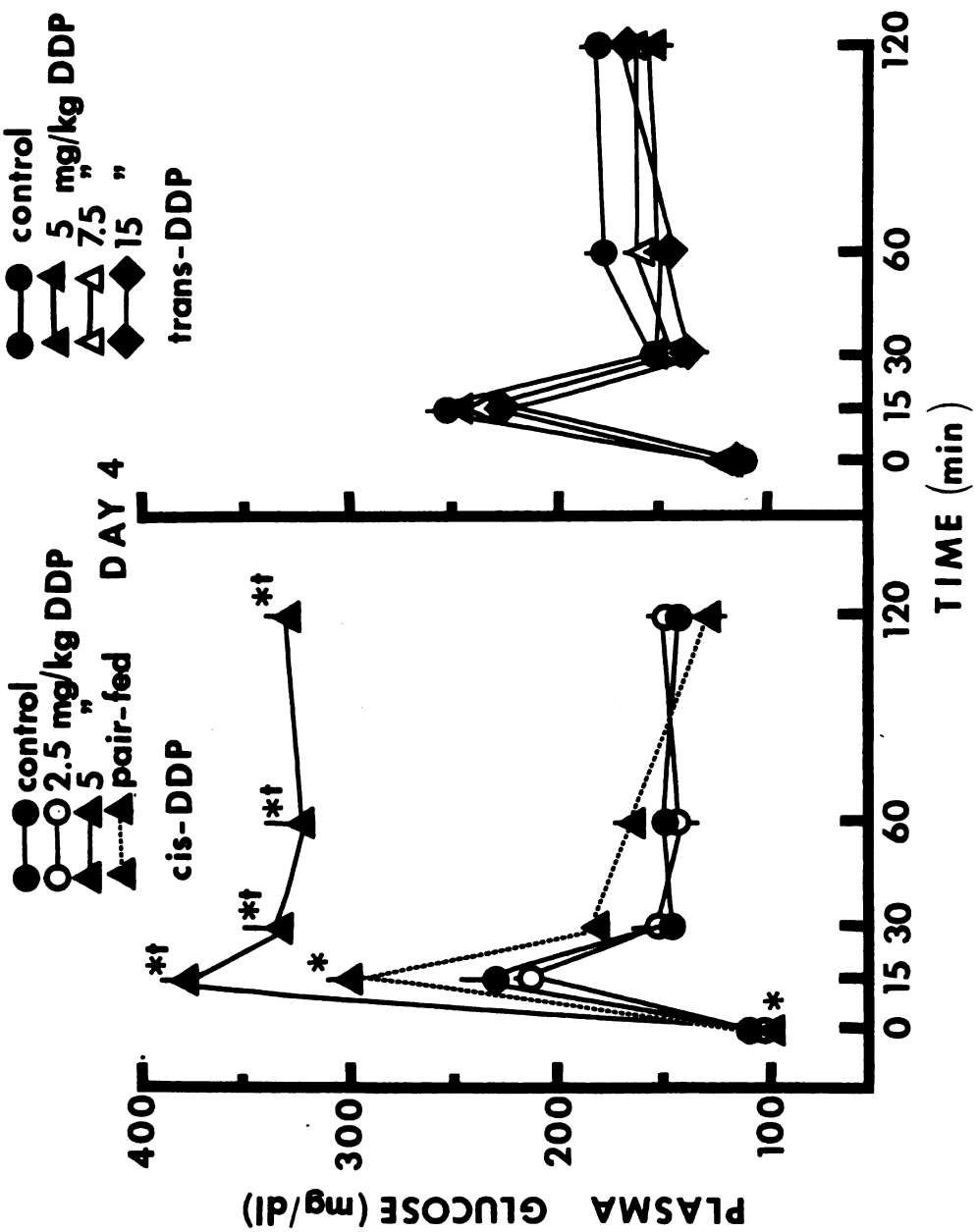
control
2.5 mg/kg DDP
5 "
pair-fed
cis-DDP
DAY 4
control
5 mg/kg DDP
7.5 "
15 "
trans-DDP
PLASMA GLUCOSE (mg/dl)
400
300
200
100
0 15 30 60 120
0 15 30 60 120
TIME (min)

Figure 12

Figure 12. Effect of cis-DDP (left) and trans-DDP (right) on glucose tolerance four days following DDP administration. Plasma glucose concentration is plotted against time following a glucose load (2 g/kg, i.p.). Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, at the corresponding time point ($p<0.05$).

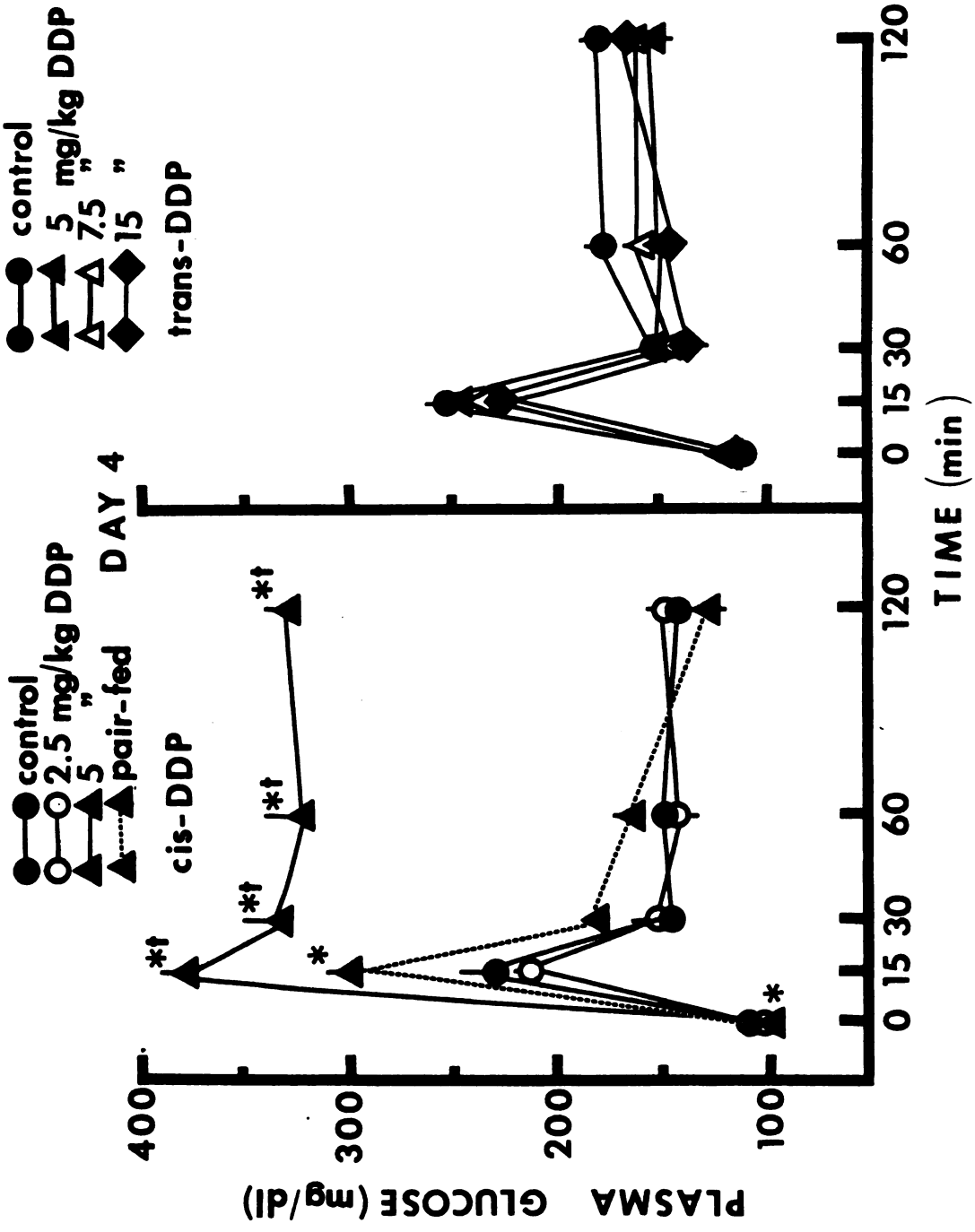
control
2.5 mg/kg DDP
5 "
pair-fed
cis-DDP
DAY 4
control
5 mg/kg DDP
7.5 "
15 "
trans-DDP
PLASMA GLUCOSE (mg/dl)
400
300
200
100
0 15 30 60 120
TIME (min)
0 15 30 60 120
TIME (min)

Figure 12

On day 7, hyperglycemia was only apparent 60 and 120 minutes following a glucose load in the 5 mg/kg cis-DDP group (Figure 13). Administration of trans-DDP did not affect glucose tolerance on this day (Figure 13). On day 14, no effect of cis-DDP, trans-DDP or reduced food intake on glucose tolerance was evident (Figure 14). In addition, administration of 2.5 mg/kg cis-DDP did not affect glucose tolerance at any time examined, i.e., days 2 (Figure 11), 4 (Figure 12), 7 (Figure 13) or 14 (Figure 14).

Plasma glucose concentration was markedly elevated fifteen minutes following a glucose load in animals treated with 18 mg/kg ammonium tetrachloroplatinate on day 2 (Figure 15). However, hyperglycemia was not evident in these animals 30, 60 and 120 minutes following glucose administration (Figure 15). No effect on glucose tolerance was apparent 4 (Figure 15), 7 or 14 (Figure 16) days following 18 mg/kg tetrachloroplatinate treatment. Administration of 6 mg/kg tetrachloroplatinate did not alter glucose tolerance at any time examined throughout the 14 day experiment (Figures 15 and 16).

Effect of cis-DDP on Plasma IRI and IRG

Fasting plasma IRI concentrations of cis-DDP treated animals (2.5 or 5 mg/kg) were comparable to ad-libitum fed controls on days 2 (Figure 17), 4 (Figure 18), 7 (Figure 19) and 14 (Figure 20). However, fasting plasma IRI concentrations of the 5 mg/kg group were significantly greater than their pair-fed partners on days 2 (Figure 17), 7 (Figure 19) and 14 (Figure 20). Following glucose stimulation, plasma IRI of all drug treated animals was comparable to ad-libitum fed controls at all times

Figure 13. Effect of cis-DDP (left) and trans-DDP (right) on glucose tolerance seven days following DDP administration. Plasma glucose concentration is plotted against time following a glucose load (2 g/kg, i.p.). Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, at the corresponding time point ($p<0.05$).

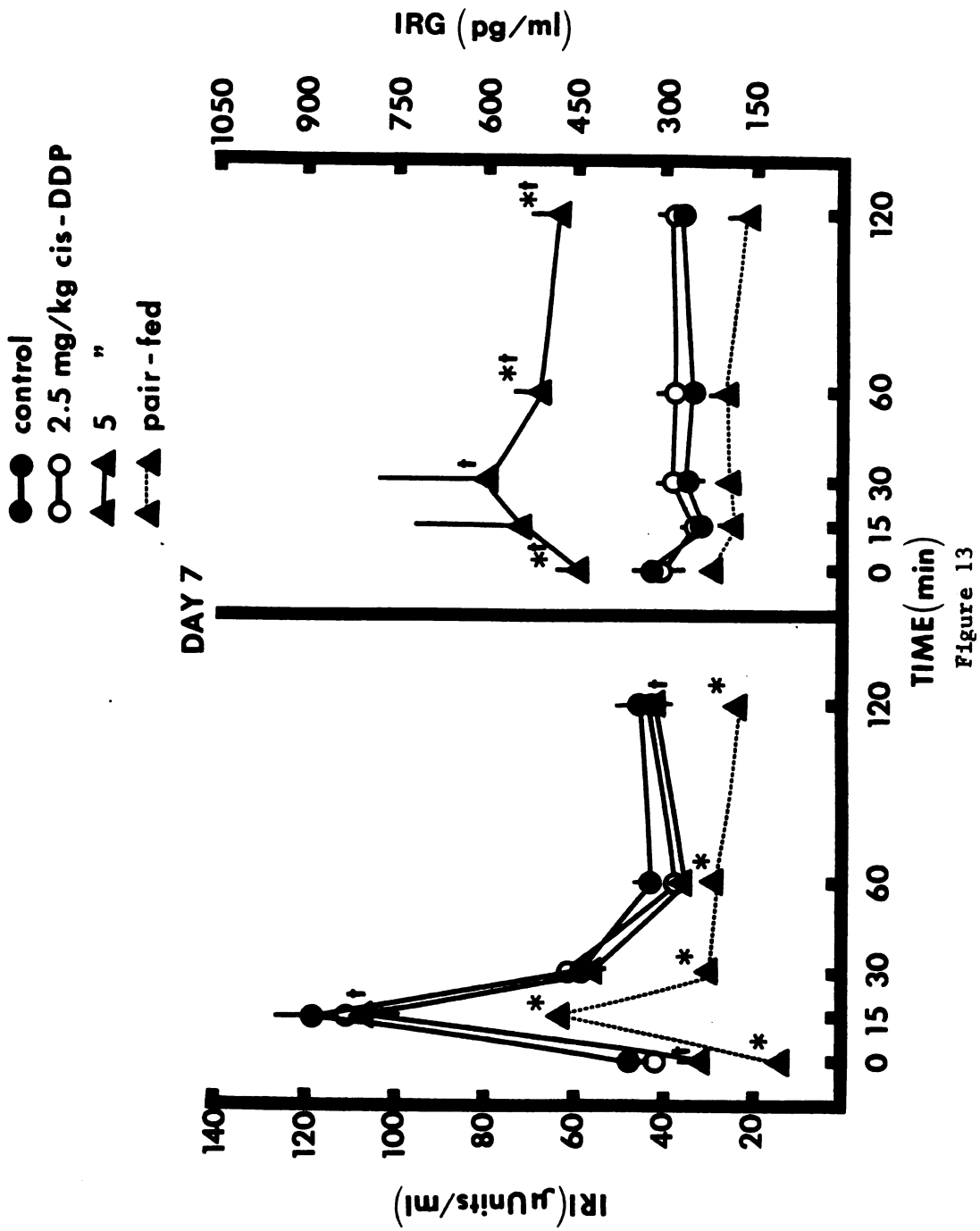
control
2.5 mg/kg cis-DDP
5 "
pair-fed
IRG (pg/ml)
1050
900
750
600
450
300
150
DAY 7
IRI(μUnits/ml)
140
120
100
80
60
40
20
0 15 30 60 120
0 15 30 60 120
TIME(min)

Figure 13

Figure 14. Effect of cis-DDP (left) and trans-DDP (right) on glucose tolerance fourteen days following DDP administration. Plasma glucose concentration is plotted against time following a glucose load (2 g/kg, i.p.). Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, at the corresponding time point ($p<0.05$).

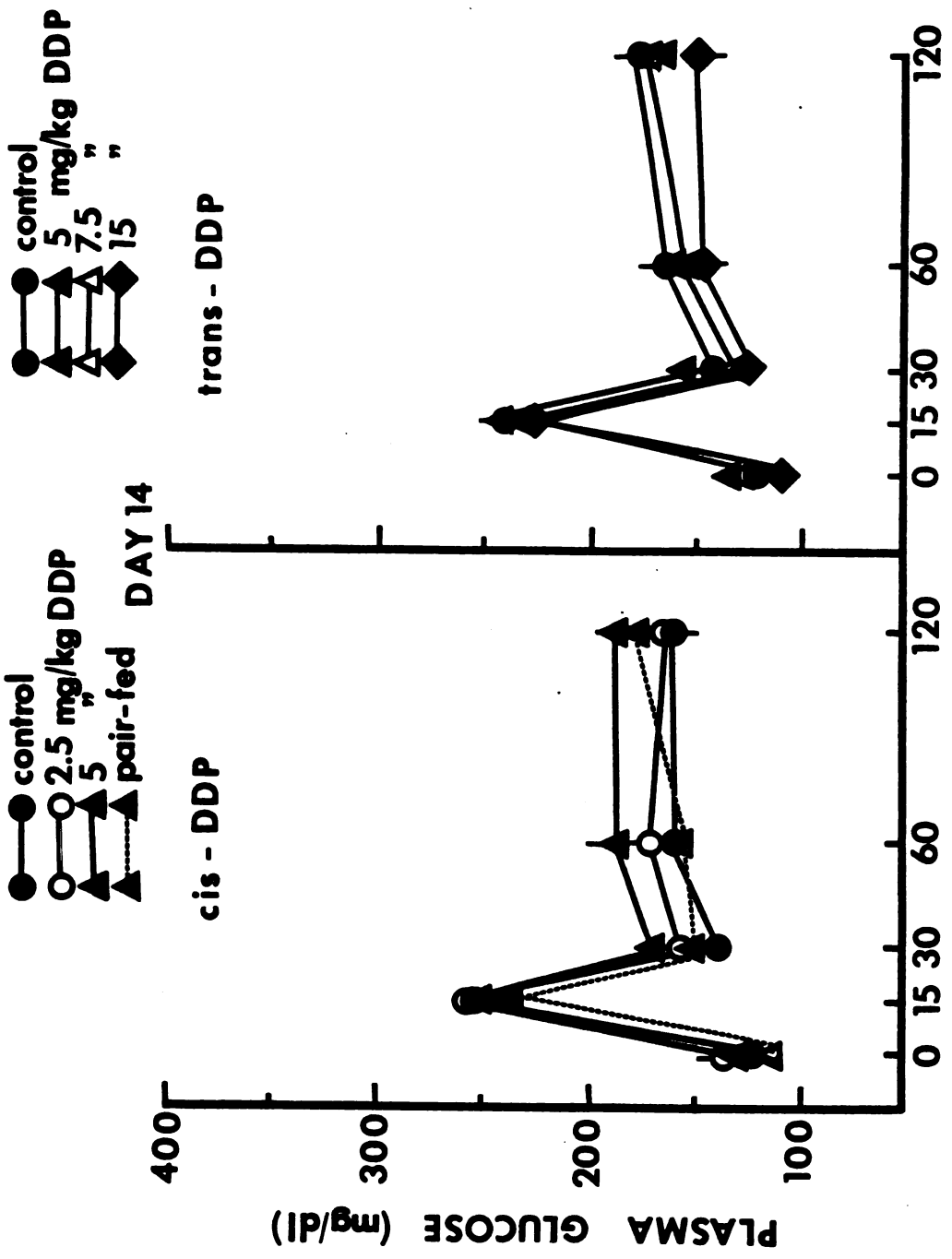
control
2.5 mg/kg DDP
5 "
pair-fed
cis - DDP
DAY 14
control
5 mg/kg DDP
7.5 "
15 "
trans - DDP
PLASMA GLUCOSE (mg/dl)
400
300
200
100
0 15 30 60 120
0 15 30 60 120
TIME (min)

Figure 14

Figure 15. Effect of ammonium tetrachloroplatinate on glucose tolerance two (left) and four (right) days following treatment. Plasma glucose concentration is plotted against time following a glucose load (2 g/kg, i.p.). Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks indicate a significant difference from controls at the corresponding time point ($p<0.05$).

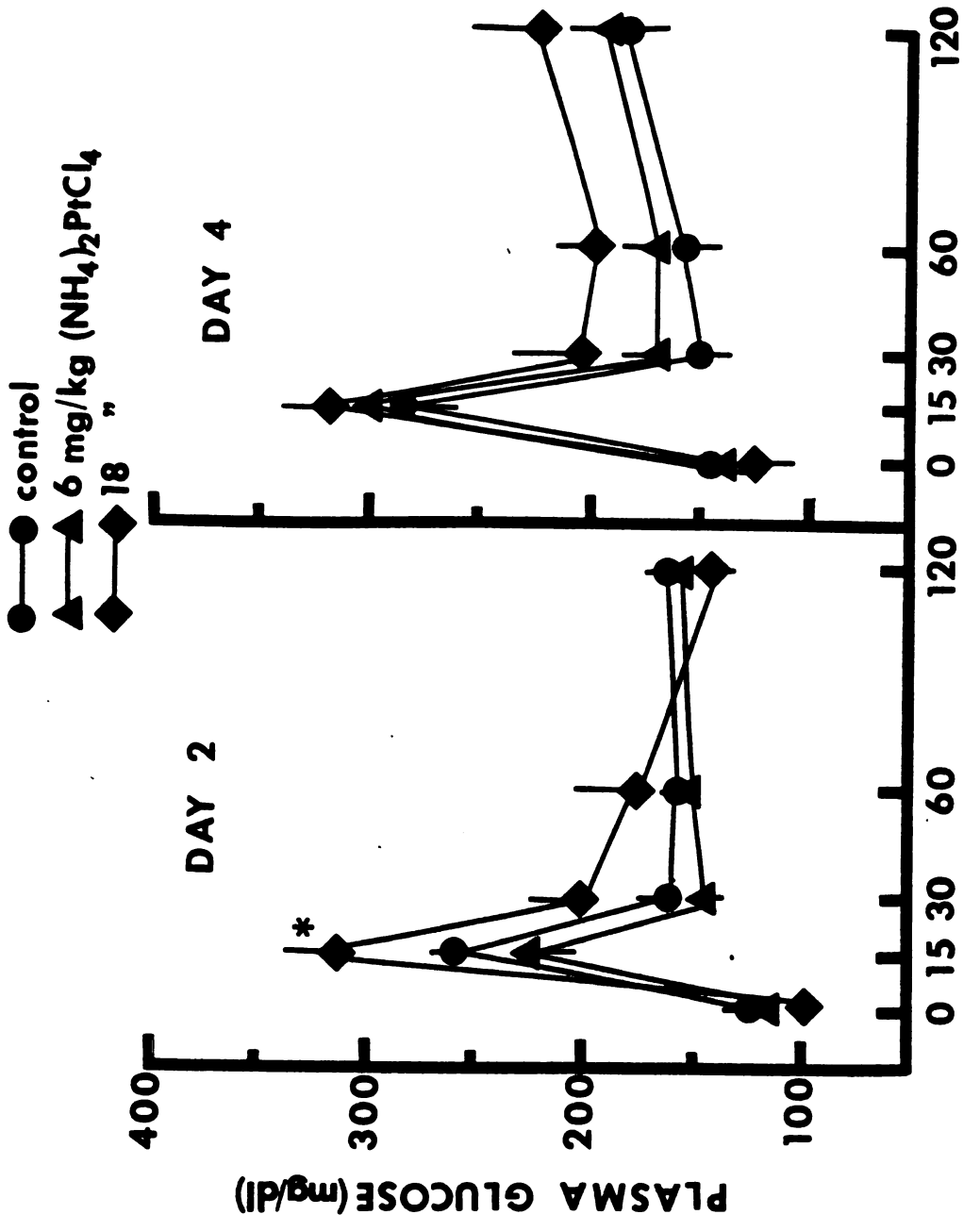
control
6 mg/kg $(NH_4)_2PtCl_4$
18 "
DAY 2
DAY 4
*
400
300
200
100
PLASMA GLUCOSE (mg/dl)
0 15 30 60 120
0 15 30 60 120
TIME (min)

Figure 15

Figure 15. Effect of ammonium tetrachloroplatinate on glucose tolerance two (left) and four (right) days following treatment. Plasma glucose concentration is plotted against time following a glucose load (2 g/kg, i.p.). Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks indicate a significant difference from controls at the corresponding time point ($p<0.05$).

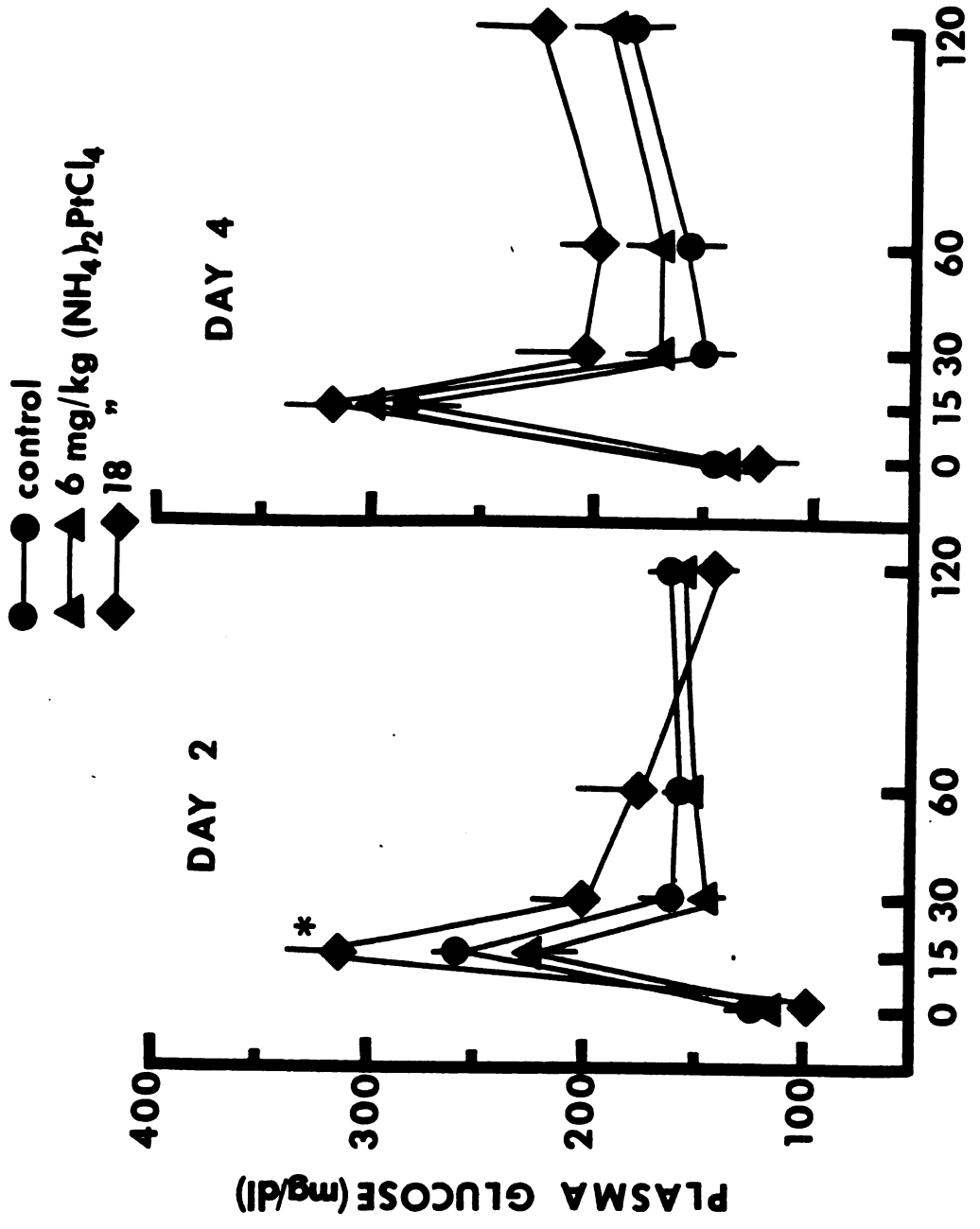
control
6 mg/kg $(NH_4)_2PtCl_4$
18 "
DAY 2
DAY 4
*
PLASMA GLUCOSE (mg/dl)
400
300
200
100
0 15 30 60 120
0 15 30 60 120
TIME (min)

Figure 15

Figure 16. Effect of ammonium tetrachloroplatinate on glucose tolerance seven (left) and fourteen (right) days following treatment. Plasma glucose concentration is plotted against time following a glucose load (2 g/kg, i.p.). Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks indicate a significant difference from controls at the corresponding time point ($p<0.05$).

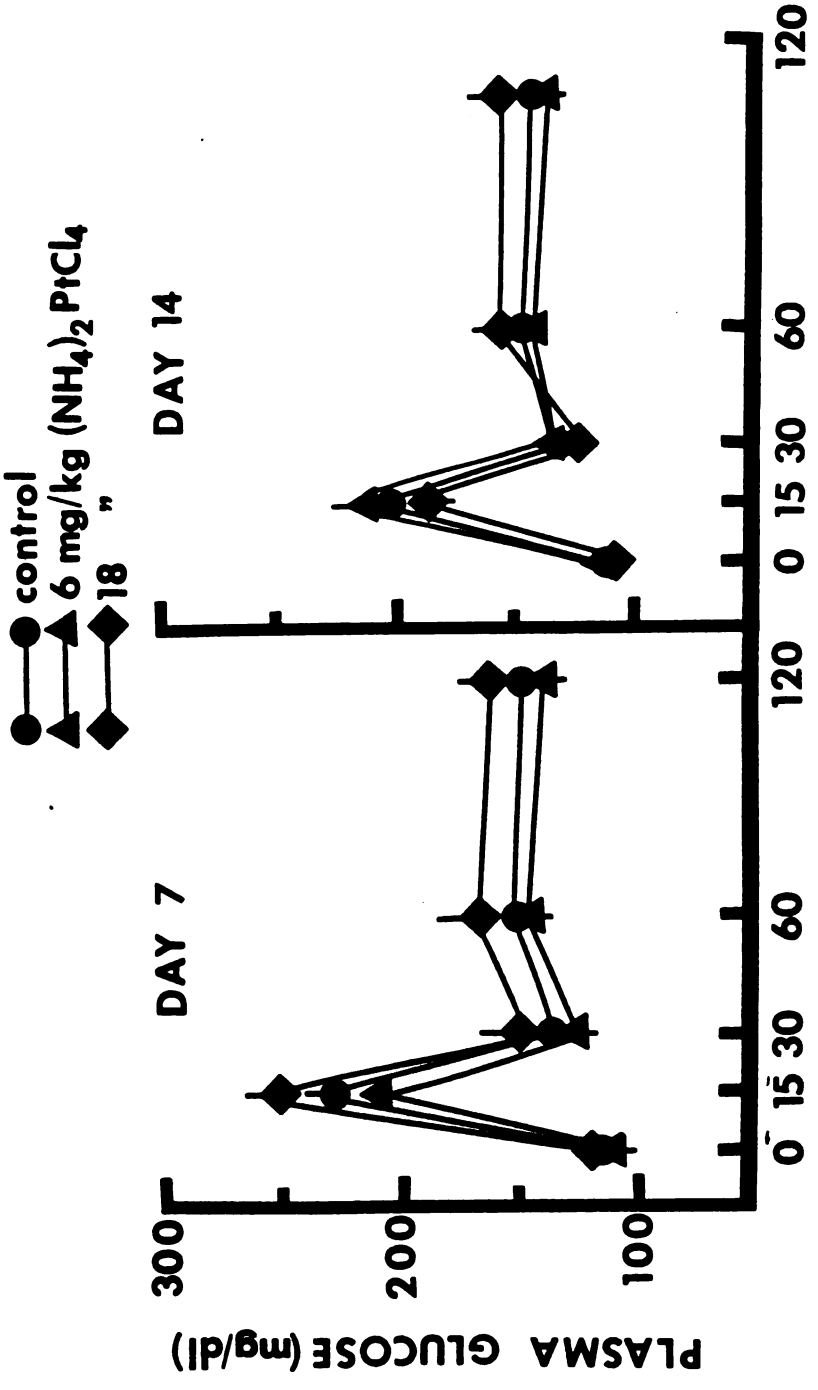
control
6 mg/kg $(NH_4)_2PtCl_4$
18 "
DAY 7
DAY 14
PLASMA GLUCOSE (mg/dl)
300
200
100
0 15 30 60 120 0 15 30 60 120
TIME (min)

Figure 16

Figure 17. Effect of cis-DDP on plasma immunoreactive insulin (IRI) (left) and glucagon (IRG) (right) two days following treatment. Plasma IRI and IRG concentrations were determined in the fasting state (0) and 15, 30, 60, and 120 minutes following a glucose load (2 g/kg, i.p.). Pair-fed animals were offered the measured amount of food consumed by the 5 mg/kg group. Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, at the corresponding time point ($p<0.05$).

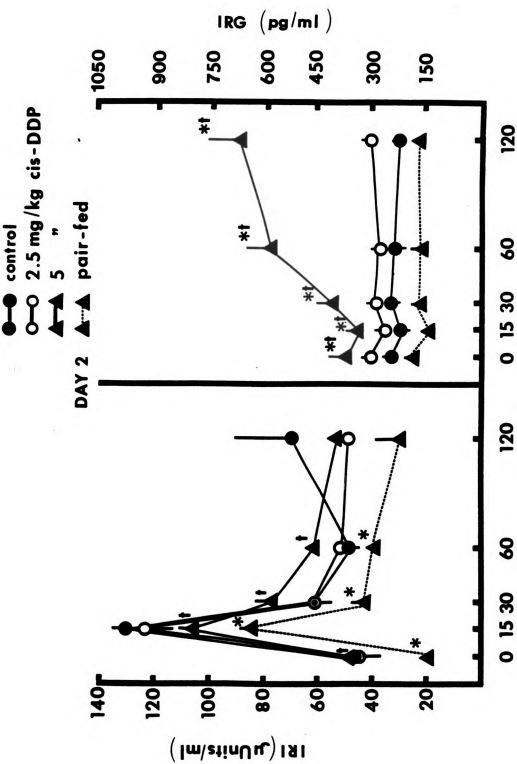
control
2.5 mg/kg cis-DDP
5 "
pair-fed
DAY 2
IRI (μUnits/ml)
IRG (pg/ml)
TIME (min)
0 15 30 60 120
0 15 30 60 120
20 40 60 80 100 120 140
150 300 450 600 750 900 1050

Figure 17

Figure 18. Effect of cis-DDP on plasma immunoreactive insulin (IRI) (left) and glucagon (IRG) (right) four days following drug treatment. Plasma IRI and IRG concentrations were determined in the fasting state (0) and 15, 30, 60, and 120 minutes following a glucose load (2 g/kg, i.p.). Pair-fed animals were offered the measured amount of food consumed by the 5 mg/kg group. Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, at the corresponding time point ($p<0.05$).

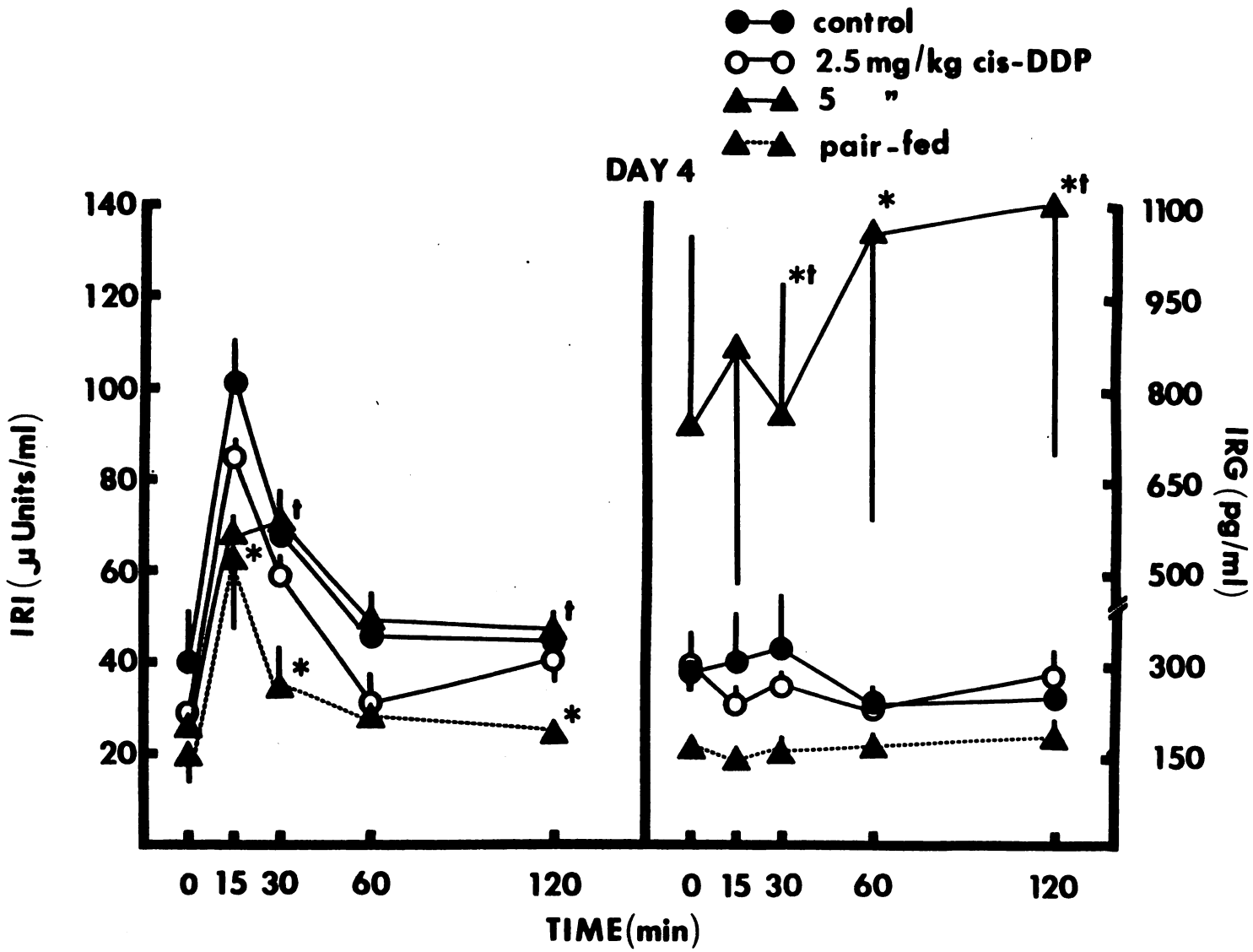
control
2.5 mg/kg cis-DDP
5 "
pair-fed
DAY 4
IRI (μ Units/ml)
IRG (pg/ml)
140
120
100
80
60
40
20
1100
950
800
650
500
300
150
0 15 30 60 120
0 15 30 60 120
TIME(min)

Figure 18

Figure 19. Effect of cis-DDP on plasma immunoreactive insulin (IRI) (left) and glucagon (IRG) (right) seven days following drug treatment. Plasma IRI and IRG concentrations were determined in the fasting state (0) and 15, 30, 60, and 120 minutes following a glucose load (2 g/kg, i.p.). Pair-fed animals were offered the measured amount of food consumed by the 5 mg/kg group. Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, at the corresponding time point ($p<0.05$).

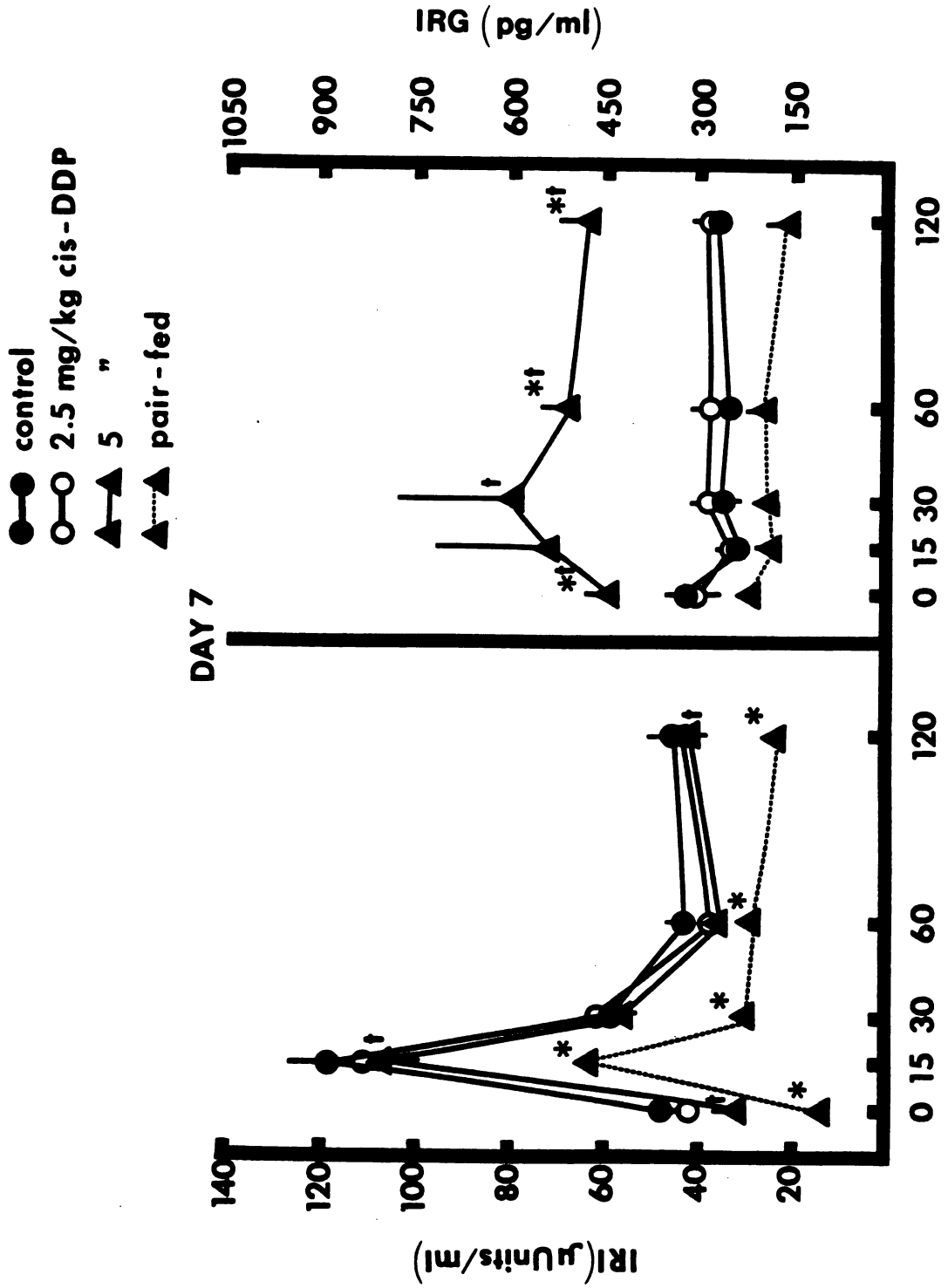
control
2.5 mg/kg cis-DDP
5 "
pair-fed
IRI (μUnits/ml)
IRG (pg/ml)
DAY 7
TIME (min)
0 15 30 60 120
0 15 30 60 120
140
120
100
80
60
40
20
1050
900
750
600
450
300
150

Figure 19

Figure 20. Effect of cis-DDP on plasma immunoreactive insulin (IRI) (left) and glucagon (IRG) (right) fourteen days following drug treatment. Plasma IRI and IRG concentrations were determined in the fasting state (0) and 15, 30, 60, and 120 minutes following a glucose load (2 g/kg, i.p.). Pair-fed animals were offered the measured amount of food consumed by the 5 mg/kg group. Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks and daggers indicate a significant difference from ad-libitum and pair-fed controls, respectively, at the corresponding time point ($p<0.05$).

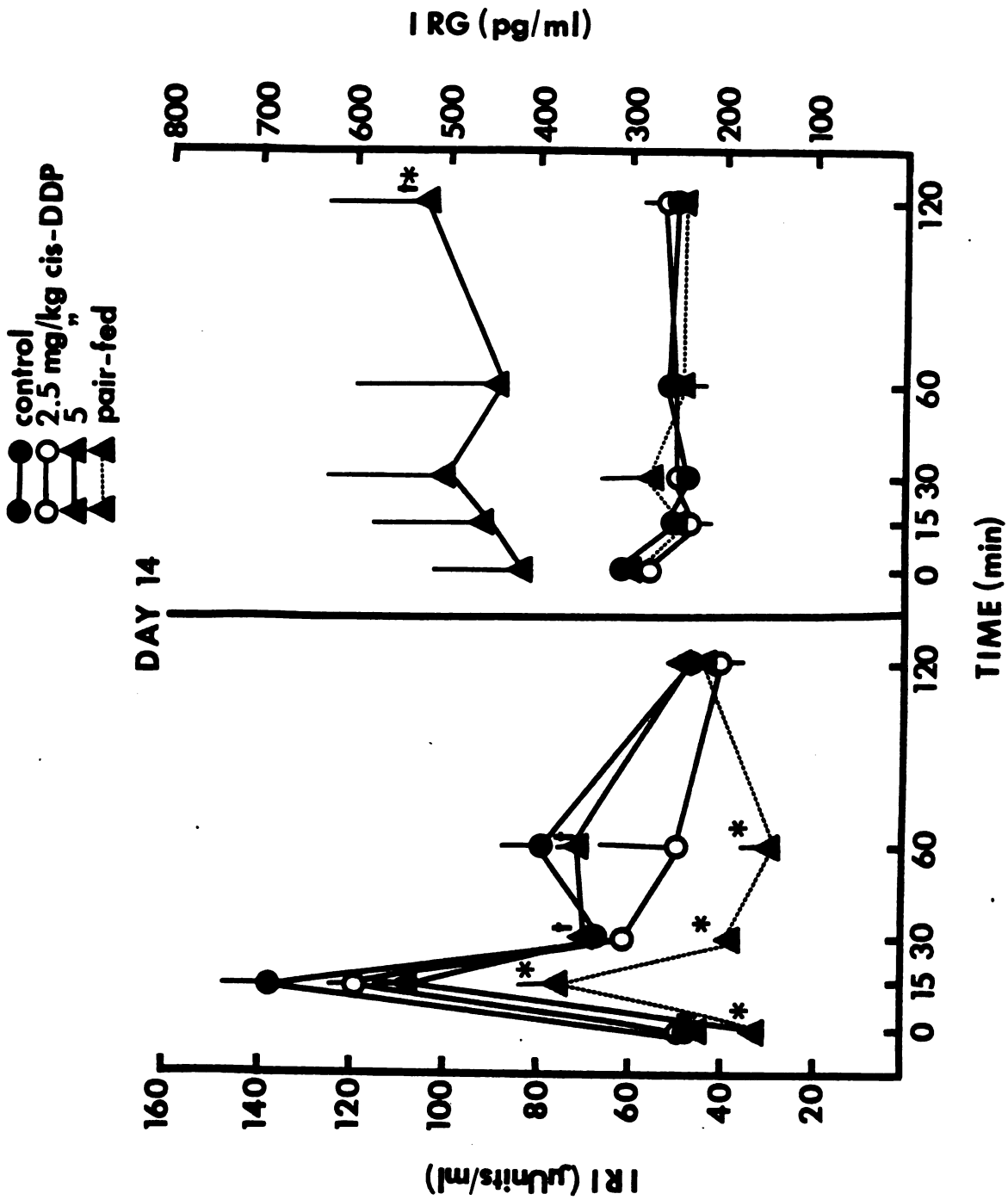
IRG (pg/ml)
800
700
600
500
400
300
200
100
control
2.5 mg/kg cis-DDP
5 "
pair-fed
DAY 14
IRI (μUnits/ml)
160
140
120
100
80
60
40
20
0 15 30 60 120
0 15 30 60 120
TIME (min)

Figure 20

examined throughout the 14 day experiment (Figures 17-20). Plasma IRI concentrations of the 5 mg/kg group, however, were significantly elevated compared to pair-fed partners following glucose stimulation. This effect was apparent on days 2 (15, 30 and 60 min) (Figure 17), 4 (30 and 120 min) (Figure 18), 7 (15 and 120 min) (Figure 19) and 14 (15, 30 and 60 min) (Figure 20).

Plasma IRI concentration of pair-fed controls was significantly diminished compared to ad-libitum fed controls 15 minutes following glucose stimulation on days 2 (Figure 17), 4 (Figure 18), 7 (Figure 19) and 14 (Figure 20). However, the percent plasma IRI response from the fasting to the glucose stimulated state (at 15 minutes) was not significantly impaired in pair-fed animals on these days (Table 3).

Although fasting plasma IRG concentrations in the 5 mg/kg group tended to be elevated on all days examined, significant differences were only observed on days 2 (Figure 17) and 7 (Figure 19). On day 2, fasting plasma IRG of animals treated with 5 mg/kg cis-DDP averaged 364±22 pg/ml compared to ad-libitum fed and pair-fed control values of 253±30 and 185±9 pg/ml, respectively (Figure 17). Although plasma IRG concentrations of the 5 mg/kg group tended to be elevated throughout the two hour glucose tolerance test on all days examined, significant differen ces throughout the two hour period were only observed on day 2 (Figure 17). Treatment with 2.5 mg/kg cis-DDP did not affect plasma IRG concentrations in the fasted state or following glucose administration on any day examined (Figures 17-20).

TABLE 3

Effects of cis-DDP on % Plasma Immunoreactive Insulin (IRI) Response to Glucose[a]

cis-DDP (mg/kg)	Time After Treatment (days)			
	2	4	7	14
0	288±34[c]	289±47	252±27	290±29
2.5	260±22	292±16	274±30	251±21
5	239±35	375±38	353±37	226±20
Pair-fed[b]	477±85[d]	327±61	459±61[d]	247±43

[a]% Plasma IRI response was determined as:

$$\frac{\mu\text{Units IRI/ml (15 minutes following glucose)}}{\mu\text{Units IRI/ml (0 minutes)}} \times 100.$$

Glucose was administered at a dose of 2 g/kg, i.p.

[b]Pair-fed animals were offered the measured amount of food consumed by the 5 mg/kg group.

[c]Values are expressed as means ± SEM of four determinations. Plasma was pooled from three identically treated animals and represents one determination.

[d]Significantly different from ad libitum fed controls ($p<0.05$).

Organ Weights

Adrenal weight to body weight (AW/BW) ratios were significantly increased 4 and 14 days following treatment with 5 mg/kg cis-DDP compared to ad-libitum fed controls (Table 4); however, significant differences compared to pair-fed animals were only observed on day 4 (Table 4). In contrast, equimolar or greater than equimolar doses of trans-DDP did not affect relative adrenal weight (Table 4). Treatment with 18 mg/kg, but not 6 mg/kg ammonium tetrachloroplatinate increased AW/BW on days 4 and 14 (Table 4).

Kidney weight to body weight (KW/BW) ratios were significantly elevated 4 days, but not 14 days, following treatment with 5 mg/kg cis-DDP (Table 4). In contrast, trans-DDP did not affect KW/BW at any time examined (Table 4). Treatment with 18 mg/kg, but not 6 mg/kg, ammonium tetrachloroplatinate increased KW/BW on days 4 and 14.

Liver weight to body weight (LW/BW) ratios were not affected by treatment with cis-DDP, trans-DDP or ammonium tetrachloroplatinate at any time examined (Table 4).

Role of Adrenal Glands in cis-DDP Glucose Intolerance

cis-DDP treated animals (5 mg/kg), with intact adrenal glands, exhibited marked hyperglycemia following a glucose load on days 2 and 4 (Figure 21). Fasting plasma glucose concentration of cis-DDP treated animals was significantly reduced by bilateral adrenalectomy (Figure 21). However, plasma glucose concentrations at 15, 30 and 60 minutes following glucose administration were comparable in both cis-DDP groups (sham

TABLE 4

Effects of Platinum Treatment on Adrenal Weight/Body Weight (AW/BW), Kidney Weight/Body Weight (KW/BW) and Liver Weight/Body Weight (LW/BW)

	Day 4										
	cis-DDP (mg/kg)				trans-DDP (mg/kg)				$(NH_4)_2PtCl_4$ (mg/kg)		
	0	2.5	5	Pair-fed	0	5	7.5	15	0	6	18
$\frac{AW}{BW} \times 10^3$	.16 ±.01[a]	.17 ±.01	.23 ±.01[bc]	.16 ±.01	.19 ±.01	.16 ±.01	.18 ±.01	.19 ±.01	.16 ±.01	.17 ±.01	.20 ±.02[b]
$\frac{KW}{BW} \times 10^2$	.83 ±.03	.88 ±.02	1.01 ±.03[bc]	.93 ±.01	.85 ±.03	.79 ±.02	.80 ±.02	.80 ±.02	.80 ±.02	.83 ±.03	.89 ±.02[b]
$\frac{LW}{BW} \times 10^2$	3.84 ±.14	3.79 ±.10	3.55 ±.10	3.07 ±.10	3.64 ±.05	3.58 ±.06	3.70 ±.08	3.60 ±.11	3.55 ±.11	3.48 ±.07	3.67 ±.08

[a]Values are expressed as means ± SEM of 8-10 animals. Pair-fed animals were offered the amount of food consumed by the 5 mg/kg cis-DDP group. [b]Significantly different from ad-libitum fed controls ($p<0.05$). [c]Significantly different from pair-fed controls ($p<0.05$).

TABLE 4 (continued)

	Day 14						
	cis-DDP (mg/kg)				$(NH_4)_2PtCl_4$ (mg/kg)		
	0	2.5	5	Pair-fed	0	6	18
$\frac{AW}{BW} \times 10^3$	.15±.01	.15±.01	.19±.02[b]	.17±.01	.18±.01	.17±.01	.22±.01[b]
$\frac{KW}{BW} \times 10^2$	.86±.02	.86±.04	.85±.02	.87±.01	.75±.01	.74±.01	.80±.01[b]
$\frac{LW}{BW} \times 10^2$	3.74±.08	3.65±.08	3.74±.11	3.37±.10	3.37±.18	3.37±.07	3.54±.06

[a]Values are expressed as means ± SEM of 8-10 animals. Pair-fed animals were offered the amount of food consumed by the 5 mg/kg cis-DDP group. [b]Signi ficantly different from ad-libitum fed controls ($p<0.05$). [c]Significantly different from pair-fed controls ($p<0.05$).

Figure 21. Effect of bilateral adrenalectomy (Adx) on cis-DDP induced glucose intolerance. Adrenalectomies were performed seven days prior to drug (5 mg/kg cis-DDP) or vehicle (saline) administration. Sham operations were performed by inspecting but not excising adrenal glands. Glucose tolerance was evaluated 2 (left) and 4 (right) days following cis-DDP or vehicle administration. Plasma glucose concentration is plotted against time after a glucose load (2 g/kg, i.p.). Each point with vertical line represents mean ± SEM of four determinations. Plasma pooled from three identically treated rats was used for one determination. Adx/Control animals did not survive the two hour glucose tolerance test on day 2. Asterisks indicate a significant difference from Adx/Controls at the corresponding time point. Daggers indicate a significant difference from Sham/cis-DDP animals at the corresponding time point ($p<0.05$).

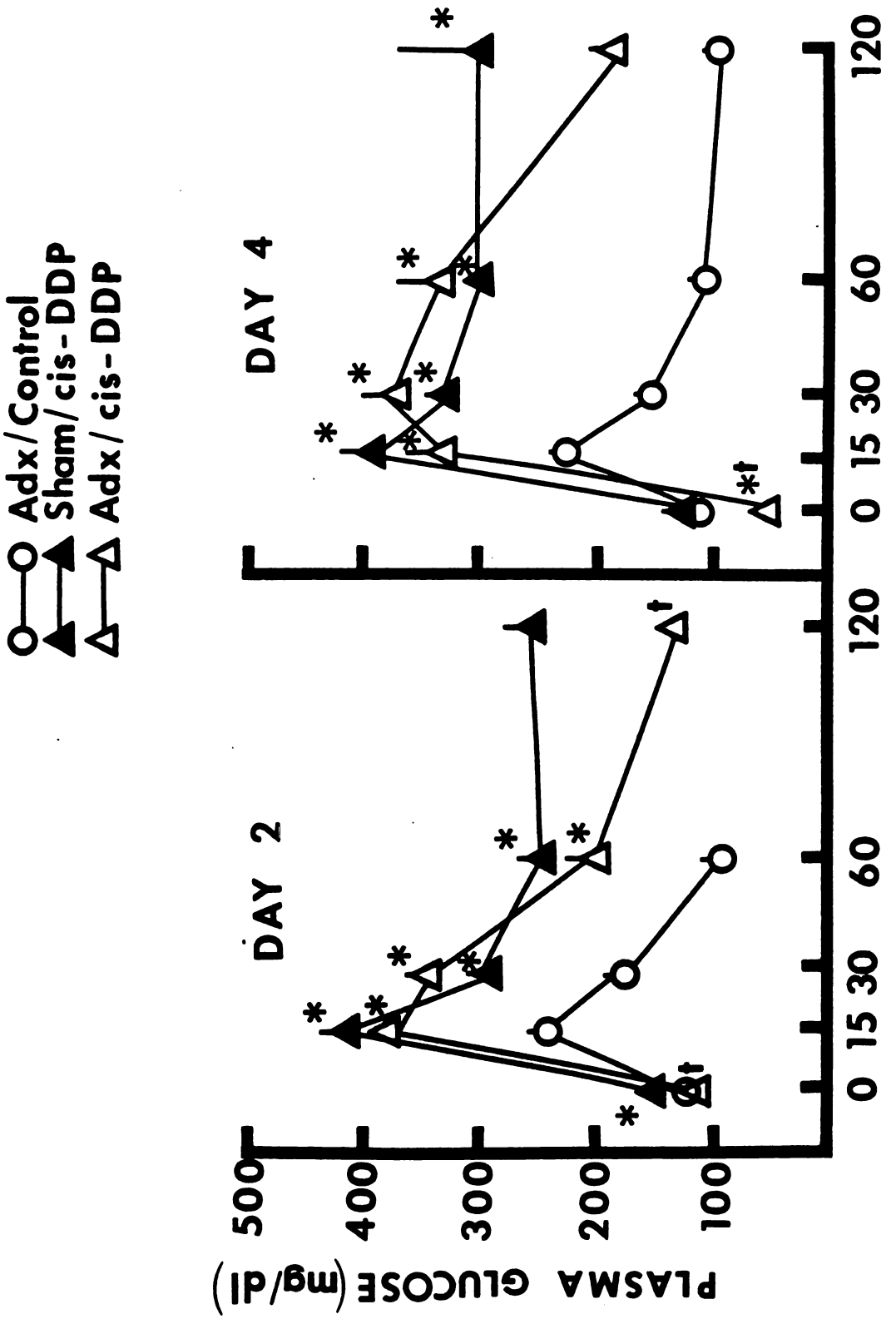
Adx/Control
Sham/cis-DDP
Adx/cis-DDP
DAY 2
DAY 4
PLASMA GLUCOSE (mg/dl)
500
400
300
200
100
0 15 30 60 120 0 15 30 60 120
TIME (min)

Figure 21

and adrenalectomized) and were each significantly elevated compared to controls (Figure 21). On day 2, plasma glucose concentrations of the Adx/cis-DDP group returned to fasting levels by 120 minutes, an effect which was not observed in sham drug treated partners (Figure 21).

On day 2, plasma IRI of cis-DDP treated animals in the fasting and glucose-stimulated state (at 15 minutes) was significantly reduced by bilateral adrenalectomy (Table 5). However, the percent increase in plasma IRI from the fasting to the glucose stimulated state (15 minutes) was not significantly different between adrenalectomized and sham treated animals receiving cis-DDP (Table 5). Neither cis-DDP treated groups (sham and adrenalectomized) exhibited hyperglucagonemia (Table 5).

Role of the Kidneys in cis-DDP Glucose Intolerance

Renal Function Following Platinum Treatment

Urine Volume and Fluid Intake. Twenty-four hour urine volume was increased 2-3 fold one and two days following treatment with 7.5 or 15 mg/kg cis-DDP and persisted through day 2 (Figure 22). Polyuria was also evident in the 5 mg/kg cis-DDP group on day 2, 4, 7 and 14 (Figure 22). Fluid intake was increased in the latter treatment group only on days 4, 7 and 14 (Figure 22). In contrast, equimolar doses of trans-DDP did not affect daily urine volume or water intake (Figure 23). Treatment with 18 mg/kg tetrachloroplatinate, however, resulted in a 3-4 fold elevation in daily urine volume on days 1, 2, and 4 (Figure 23). Water intake was also increased in these animals 2 and 4 days following platinum treatment (Figure 23). Tetrachloroplatinate (18 mg/kg) did not

and adrenalectomized) and were each significantly elevated compared to controls (Figure 21). On day 2, plasma glucose concentrations of the Adx/cis-DDP group returned to fasting levels by 120 minutes, an effect which was not observed in sham drug treated partners (Figure 21).

On day 2, plasma IRI of cis-DDP treated animals in the fasting and glucose-stimulated state (at 15 minutes) was significantly reduced by bilateral adrenalectomy (Table 5). However, the percent increase in plasma IRI from the fasting to the glucose stimulated state (15 minutes) was not significantly different between adrenalectomized and sham treated animals receiving cis-DDP (Table 5). Neither cis-DDP treated groups (sham and adrenalectomized) exhibited hyperglucagonemia (Table 5).

Role of the Kidneys in cis-DDP Glucose Intolerance

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TABLE 5

Effects of Bilateral Adrenalectomy (Adx) on Plasma Immunoreactive Insulin (IRI) and Glucagon (IRG) in cis-DDP Treated Rats

	Plasma IRI (μUnits/ml)			Plasma IRG (pg/ml)	
	Time After Glucose (minutes)[a]			Time After Glucose (minutes)	
Treatment	0	15	% Plasma IRI Response[e]	0	15
Adx/Control	12±2[b]	77±20	637±137	282±20	279±22
Adx/cis-DDP	13±4[c]	42± 4[c]	386±137	436±66	449±88
Sham/cis-DDP	50±7[d]	94±12	203± 48[d]	388±36	375±65

[a]Blood was sampled in the fasting state (0') and 15 minutes following a glucose load (2 g/kg, i.p.).

[b]Values are expressed as means ± SEM of four experiments. Each experiment consisted of plasma pooled from three identically treated animals. Experiments were conducted two days following cis-DDP (5 mg/kg, i.v.) or vehicle (saline) administration.

[c]Significantly different from Sham/cis-DDP ($p<0.05$).

[d]Significantly different from Adx/Controls ($p<0.05$).

[e]% Plasma IRI response determined as $\frac{\mu\text{Units IRI/ml (15 minutes)}}{\mu\text{Units IRI/ml (0 minutes)}} \times 100$.

Figure 22. Effect of cis-DDP on daily water intake (left) and urine volume (right). Each bar with vertical line represents the mean ± SEM of four to five determinations. Open bars represent pair-fed partners for each drug treatment. Animals treated with 7.5 or 15 mg/kg cis-DDP did not survive the duration of the 14 day experiment. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, on the corresponding day ($p<0.05$).

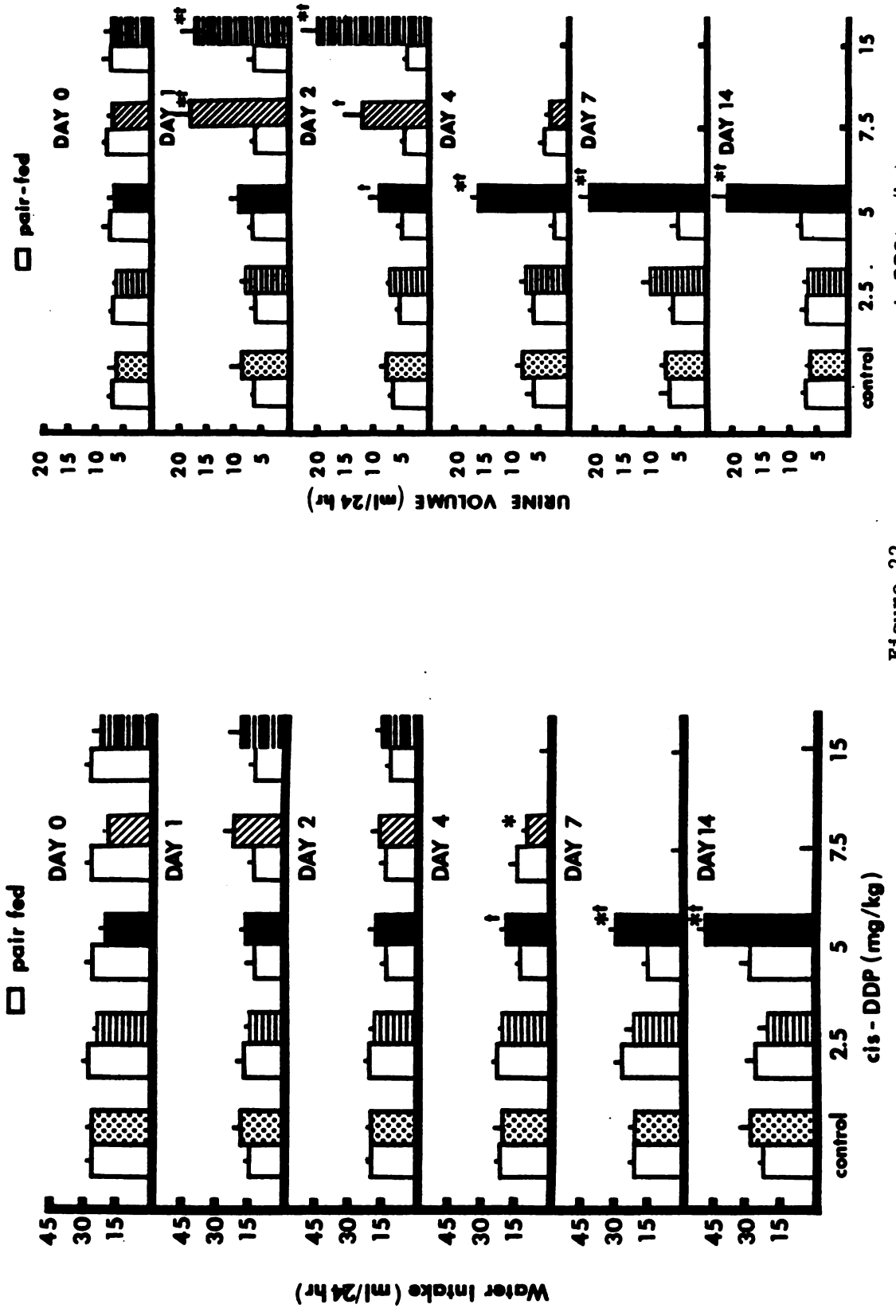
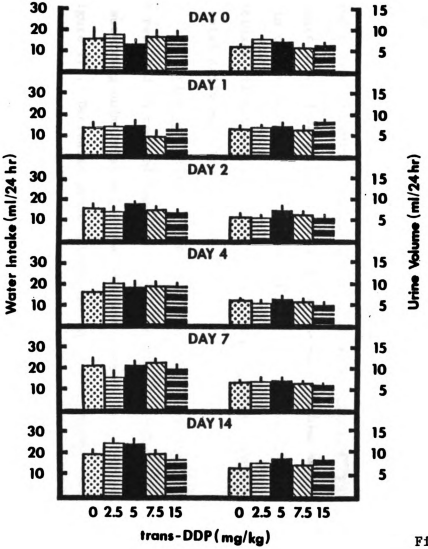

pair-fed
DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
Water Intake (ml/24 hr)
45
30
15
control
2.5
5
7.5
15
cis-DDP (mg/kg)
URINE VOLUME (ml/24 hr)
20
15
10
5

Figure 22

Figure 23. Effect of trans-DDP (left) and ammonium tetrachloroplatinate (right) on daily water intake and urine volume. Each bar with vertical line represents the mean ± SEM of four to five determinations. Identically patterned bars indicate equimolar platinum treatment. Asterisks indicate a significant difference from controls on the corresponding day ($p<0.05$).


DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
Water Intake (ml/24 hr)
Urine Volume (ml/24 hr)
0 2.5 5 7.5 15 0 2.5 5 7.5 15
trans-DDP (mg/kg)

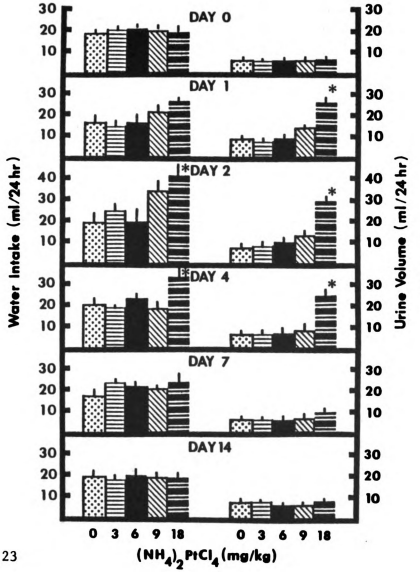

DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
Water Intake (ml/24 hr)
Urine Volume (ml/24 hr)
0 3 6 9 18 0 3 6 9 18
(NH4)2PtCl4 (mg/kg)

Figure 23

affect daily fluid intake or urine volume on days 7 and 14 (Figure 23). Smaller doses of tetrachloroplatinate did not affect daily urine volume or fluid intake at any time examined (Figure 23).

Urine Glucose. Urinary glucose concentrations were markedly elevated two days following treatment with 15 mg/kg cis-DDP and four days following treatment with 5 or 7.5 mg/kg cis-DDP (Figure 24). On days 7 and 14, urinary glucose concentrations of the 5 mg/kg cis-DDP group were comparable to controls (Figure 24). Daily urinary glucose excretion was increased in the 5, 7.5 and 15 mg/kg cis-DDP groups, averaging 241±58 mg/24 hours 4 days following administration of 5 mg/kg cis-DDP (Figure 24). Urinary glucose excretion was also increased in the 5 mg/kg group on day 7, but not on day 14 (Figure 24). In contrast, administration of equimolar doses of trans-DDP did not affect urinary glucose concentration or total daily excretion at any time examined (Figure 25).

Urinary glucose concentration and excretion was increased 1, 2 and 4 days following administration of 18 mg/kg tetrachloroplatinate (Figure 26). However, glycosuria was no longer apparent on days 7 and 14 in this treatment group (Figure 26). Smaller doses of tetrachloroplatinate did not significantly affect urinary glucose concentration or total daily excretion (Figure 26).

Urine Osmolality. Administration of 5, 7.5 or 15 mg/kg cis-DDP resulted in reduced osmolality on day 2 and this effect persisted in surviving animals at all times examined during the 14 day experiment (Figure 27). Decreased urine osmolality was also apparent in the 2.5

Figure 24. Effect of cis-DDP on concentration (left) and daily excretion (right) of urinary glucose. Each point with vertical line represents the mean ± SEM of four to five determinations. Animals treated with 7.5 or 15 mg/kg cis-DDP did not survive the duration of the 14 day experiment. The stippled area represents the mean ± 95% confidence interval of pair-fed animals. Asterisks and daggers indicate a significant difference from ad-libitum and pair-fed controls, respectively, on the corresponding day ($p<0.05$).

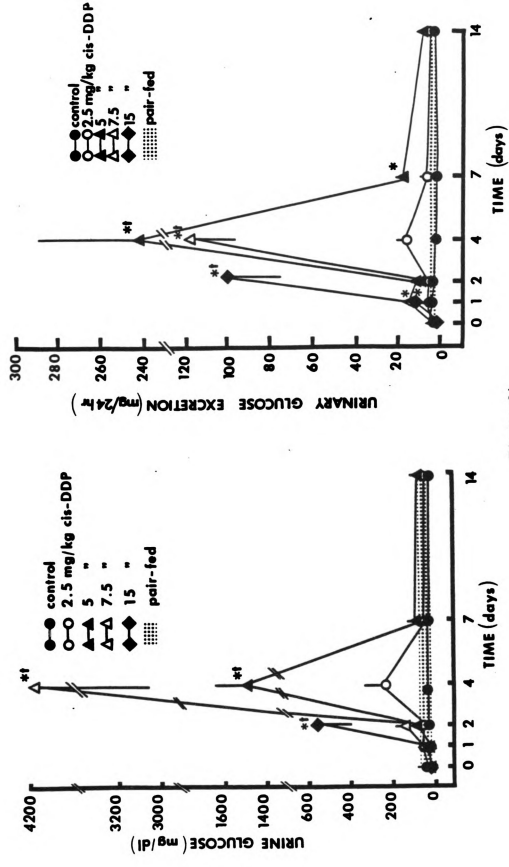

URINE GLUCOSE (mg/dl)
4200
3200
3000
1600
1400
600
400
200
0
0 1 2 4 7 14
TIME (days)
control
2.5 mg/kg cis-DDP
5 "
7.5 "
15 "
pair-fed
URINARY GLUCOSE EXCRETION (mg/24hr)
300
280
260
240
120
100
80
60
40
20
0
0 1 2 4 7 14
TIME (days)
control
2.5 mg/kg cis-DDP
5 "
7.5 "
15 "
pair-fed

Figure 24

Figure 25. Effect of trans-DDP on concentration (left) and daily excretion (right) of urinary glucose. Each bar with vertical line represents the mean ± SEM of four to five determination.

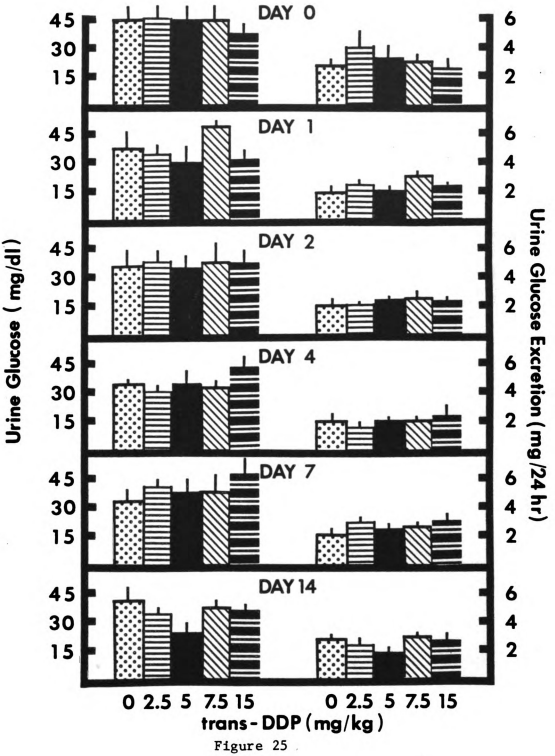

DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
45
30
15
6
4
2
Urine Glucose (mg/dl)
Urine Glucose Excretion (mg/24 hr)
0 2.5 5 7.5 15
0 2.5 5 7.5 15
trans-DDP (mg/kg)

Figure 25

Figure 26. Effect of ammonium tetrachloroplatinate on concentration (left) and daily excretion (right) of urinary glucose. Each point with vertical line represents the mean ± SEM of four to five determinations. Asterisks indicate a significant difference from controls on the corresponding day ($p<0.05$).

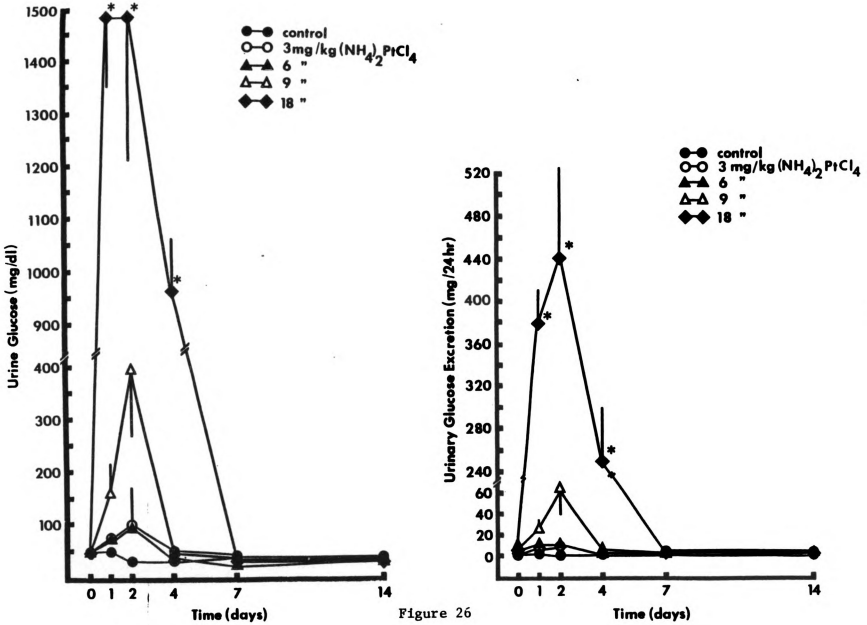

control
3mg/kg $(NH_4)_2PtCl_4$
6 "
9 "
18 "
Urine Glucose (mg/dl)
1500
1400
1300
1200
1100
1000
900
400
300
200
100
0 1 2 4 7 14
Time (days)
control
3 mg/kg $(NH_4)_2PtCl_4$
6 "
9 "
18 "
Urinary Glucose Excretion (mg/24 hr)
520
480
440
400
360
320
280
240
60
40
20
0
0 1 2 4 7 14
Time (days)

Figure 26

Figure 27. Effect of cis-DDP on urine osmolality (left) and daily urine osmolar excretion (right). Each bar with vertical line represents the mean ± SEM of four to five determinations. Open bars represent pair-fed partners for each drug treatment. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, on the corresponding day ($p<0.05$).

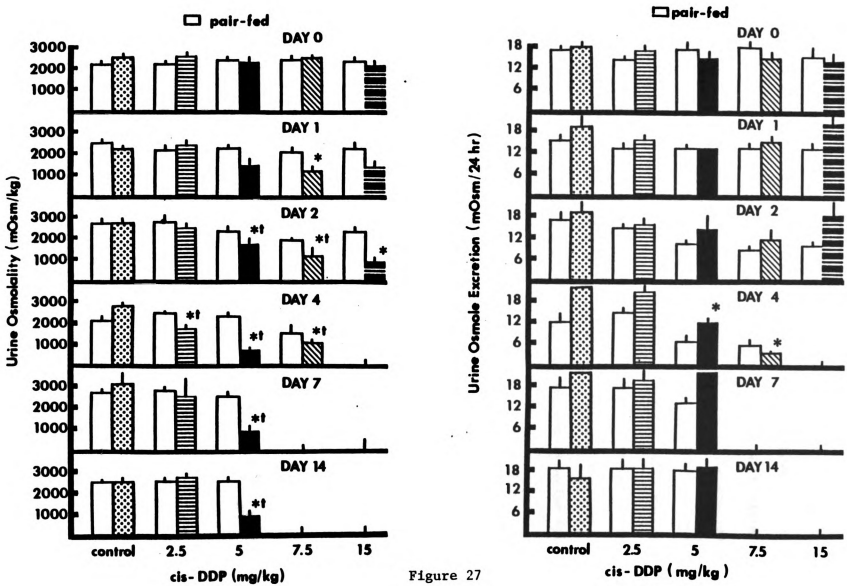

pair-fed
DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
Urine Osmolality (mOsm/kg)
3000
2000
1000
Urine Osmole Excretion (mOsm/24 hr)
18
12
6
*
*†
control
2.5
5
7.5
15
cis-DDP (mg/kg)

Figure 27

mg/kg cis-DDP group, but only 4 days following drug treatment (Figure 27). Daily urine osmolar excretion was reduced 4 days following administration of 5 or 7.5 mg/kg cis-DDP compared to ad-libitum, but not pair-fed, controls (Figure 27). In contrast, treatment with equimolar doses of the trans isomer did not alter urine osmolar concentration or daily excretion at any time examined (Figure 28). Although urine osmolality of animals treated with 18 mg/kg tetrachloroplatinate averaged 30-40% of controls on days 1, 2, 4 and 7, daily urine osmolar excretion was not affected by this treatment (Figure 28). Smaller doses of tetrachloroplatinate did not affect urine osmolar concentration or daily excretion at any time examined (Figure 28).

Sodium and Potassium. cis-DDP treatment (5, 7.5 or 15 mg/kg) resulted in decreased urinary sodium (Figure 29) and potassium (Figure 31) concentrations on day 2 and persisted in surviving animals at all times examined through day 14, with the exception of the 15 mg/kg group which exhibited increased urinary sodium concentrations on day 4 (Figure 29). Two and four days following treatment with 5, 7.5 or 15 mg/kg cis-DDP, 24 hour urinary sodium (Figure 29) and potassium (Figure 31) excretion decreased compared to ad-libitum, but not pair-fed, controls. Compared to pair-fed controls, urinary potassium excretion was markedly increased two days following treatment with 15 mg/kg (Figure 31).

Administration of 7.5 or 15 mg/kg trans-DDP resulted in reduced urinary sodium (Figure 30), but not potassium (Figure 32), concentrations only on day 2. Daily urinary sodium excretion was only significantly affected 2 days following administration of 15 mg/kg trans-DDP (Figure 30). Urinary concentration or excretion of potassium was not affected by trans-DDP treatment at any time examined (Figure 31).

Figure 28. Effect of trans-DDP (left) and ammonium tetrachloroplatinate (right) on urine osmolality and daily urine osmolar excretion. Each bar with vertical line represents the mean ± SEM of four to five determinations. Identically patterned bars indicate equimolar platinum treatment. Asterisks indicate a significant difference from controls on the corresponding day ($p<0.05$).

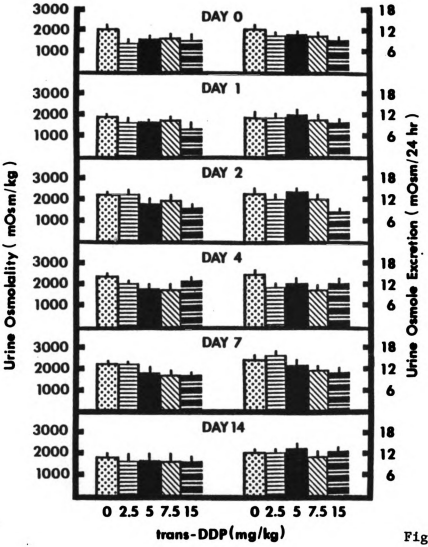

DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
3000
2000
1000
18
12
6
Urine Osmolality (mOsm/kg)
Urine Osmole Excretion (mOsm/24 hr)
0 2.5 5 7.5 15 0 2.5 5 7.5 15
trans-DDP (mg/kg)

Figure 28

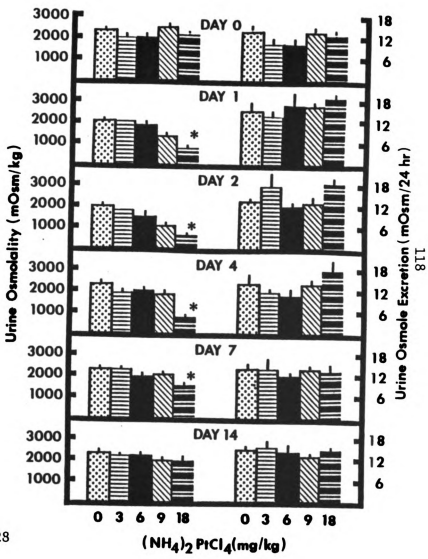

DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
*
3000
2000
1000
18
12
6
Urine Osmolality (mOsm/kg)
Urine Osmole Excretion (mOsm/24 hr)
0 3 6 9 18 0 3 6 9 18
(NH4)2 PtCl4 (mg/kg)

Figure 29. Effect of cis-DDP on concentration (left) and daily excretion (right) of urinary sodium. Each bar with vertical line represents the mean ± SEM of four to five determinations. Open bars represent pair-fed partners for each drug treatment. Animals treated with 7.5 or 15 mg/kg cis-DDP did not survive the duration of the 14 day experiment. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, on the corresponding day ($p<0.05$).


pair fed
DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
Urine Na+ Concentration (meq/l)
Urine Na+ Excretion (meq/24hr)
control
2.5
5
7.5
15
cis-DDP (mg/kg)

Figure 29

Figure 29. Effect of cis-DDP on concentration (left) and daily excretion (right) of urinary sodium. Each bar with vertical line represents the mean ± SEM of four to five determinations. Open bars represent pair-fed partners for each drug treatment. Animals treated with 7.5 or 15 mg/kg cis-DDP did not survive the duration of the 14 day experiment. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, on the corresponding day ($p<0.05$).

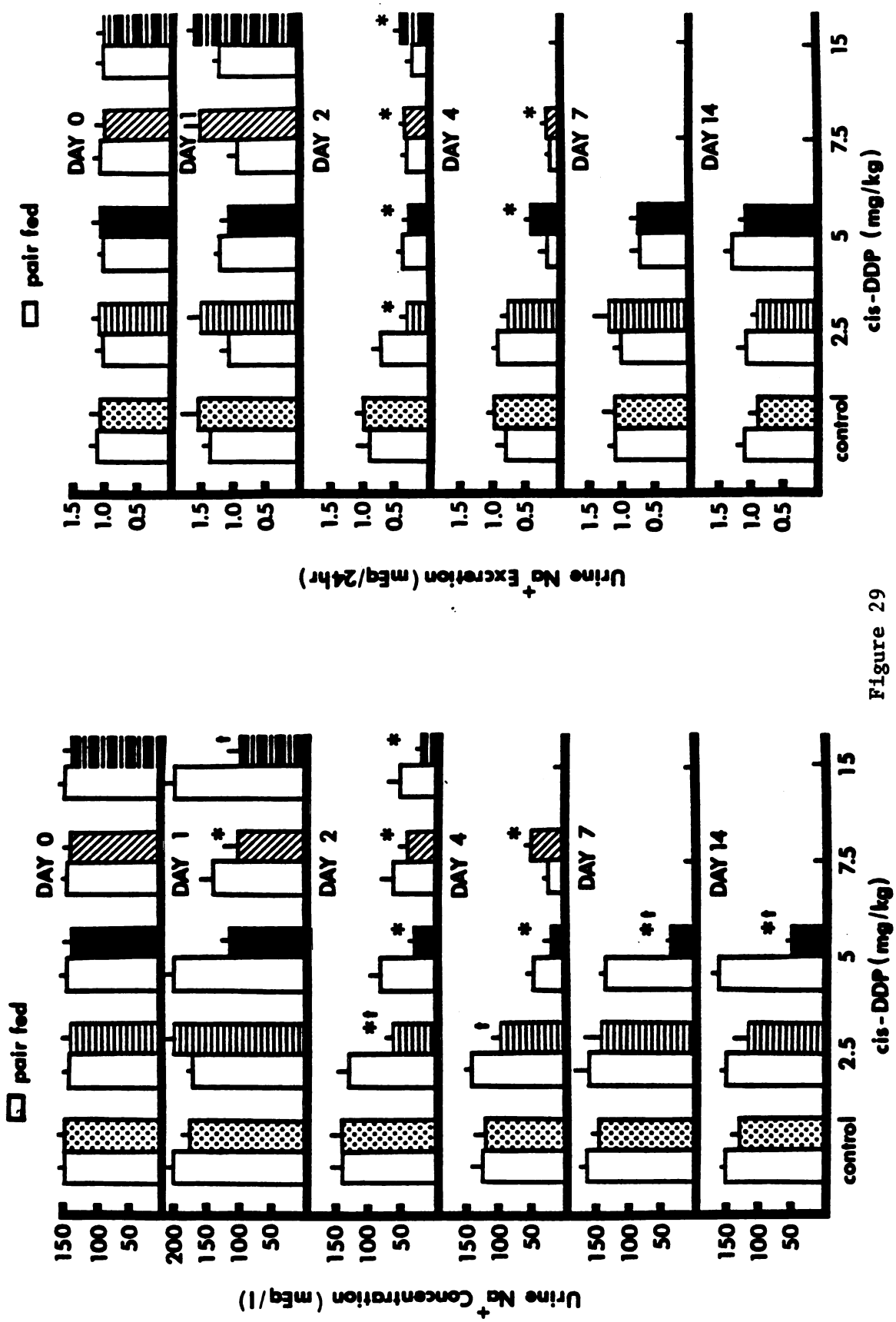

pair fed
DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
Urine Na+ Concentration (meq/l)
Urine Na+ Excretion (meq/24hr)
control
2.5
5
7.5
15
cis-DDP (mg/kg)

Figure 29

Figure 30. Effect of trans-DDP (left) and ammonium tetrachloroplatinate (right) on concentration and daily excretion of urinary sodium. Each bar with vertical line represents the mean ± SEM of four to five determinations. Identically patterned bars represent equimolar platinum treatments. Asterisks indicate a significant difference from controls on the corresponding day ($p<0.05$).

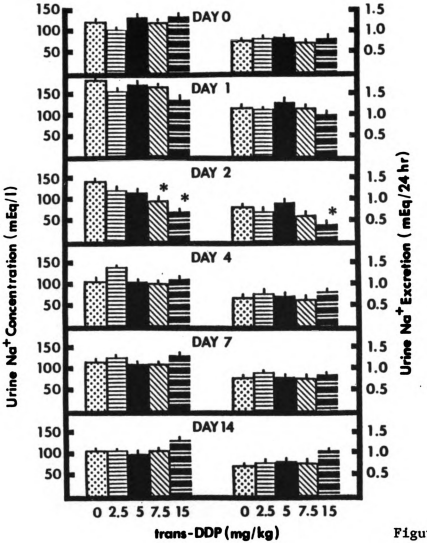

DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
150
100
50
1.5
1.0
0.5
*
0 2.5 5 7.5 15
trans-DDP (mg/kg)
Urine Na+ Concentration (mEq/l)
Urine Na+ Excretion (mEq/24 hr)

Figure 30

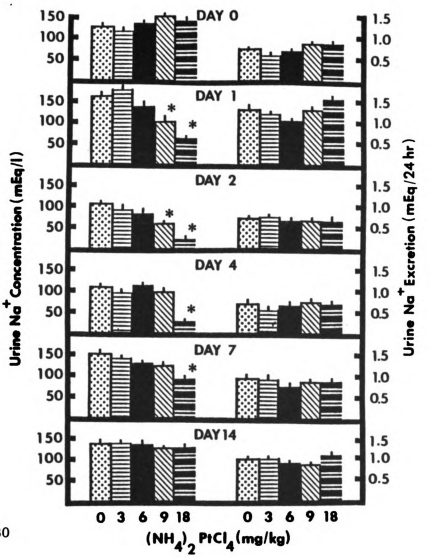

DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
150
100
50
1.5
1.0
0.5
*
0 3 6 9 18
(NH4)2 PtCl4 (mg/kg)
Urine Na+ Concentration (mEq/l)
Urine Na+ Excretion (mEq/24 hr)

Figure 31. Effect of cis-DDP on concentration (left) and daily excretion (right) of urinary potassium. Each bar with vertical line represents the mean ± SEM of four to five determinations. Open bars represent pair-fed partners for each drug treatment. Animals treated with 7.5 or 15 mg/kg cis-DDP did not survive the duration of the 14 day experiment. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively on the corresponding day ($p<0.05$).

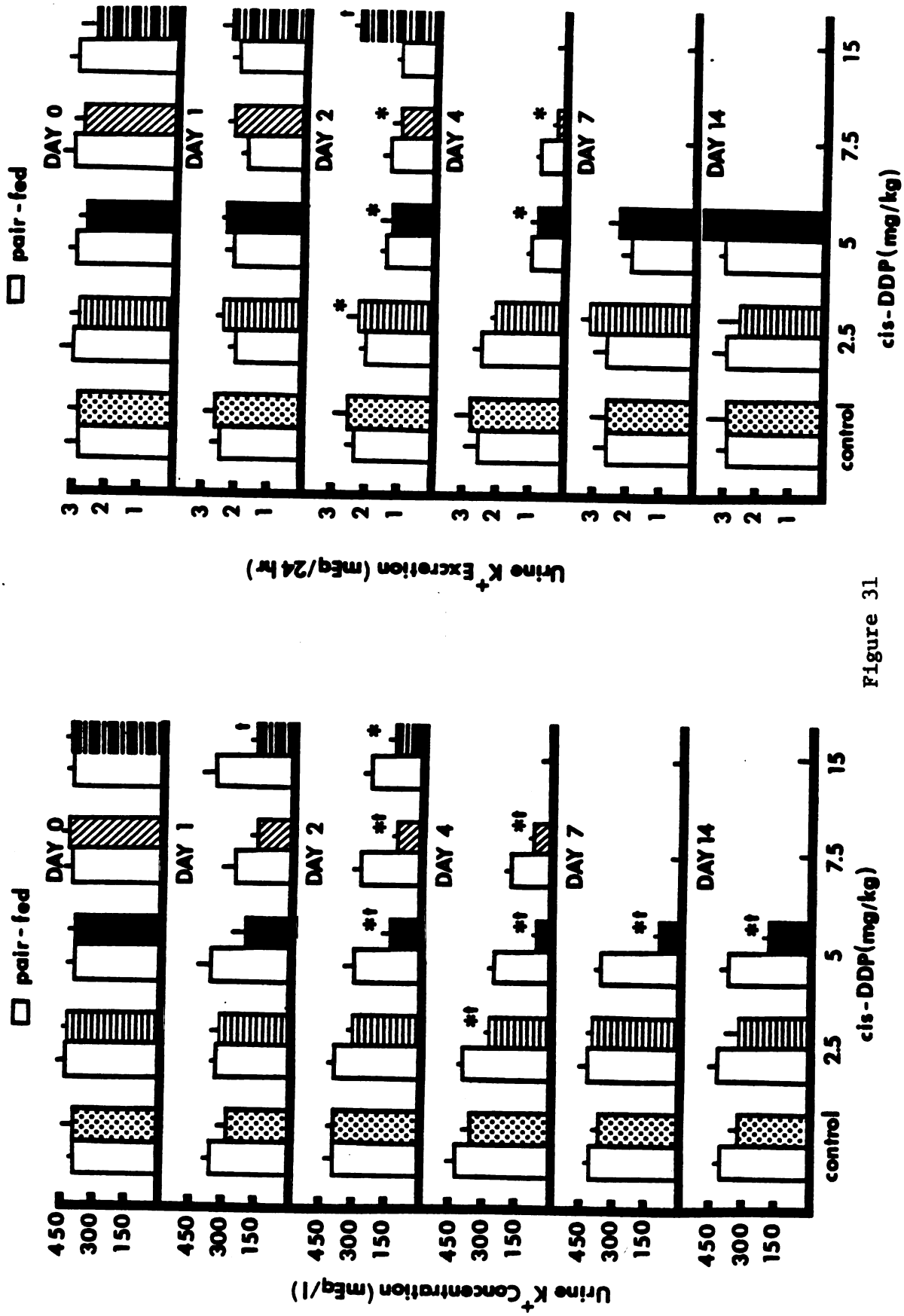
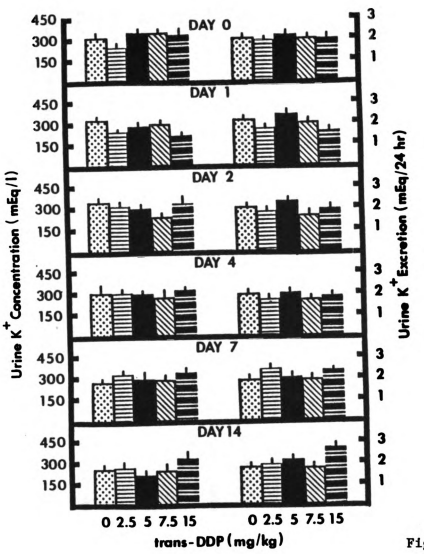

pair-fed
DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
Urine K+ Concentration (mEq/l)
Urine K+ Excretion (mEq/24 hr)
control
2.5
5
7.5
15
cis-DDP(mg/kg)

Figure 31

Figure 32. Effect of trans-DDP (left) and ammonium tetrachloroplatinate (right) on concentration and daily excretion of urinary potassium. Each bar with vertical line represents the mean ± SEM of four to five determinations. Identically patterned bars represent equimolar platinum treatment. Asterisks indicate a significant difference from controls on the corresponding day ($p<0.05$).


DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
450
300
150
3
2
1
0 2.5 5 7.5 15
0 2.5 5 7.5 15
trans-DDP (mg/kg)
Urine K+ Concentration (mEq/l)
Urine K+ Excretion (mEq/24 hr)

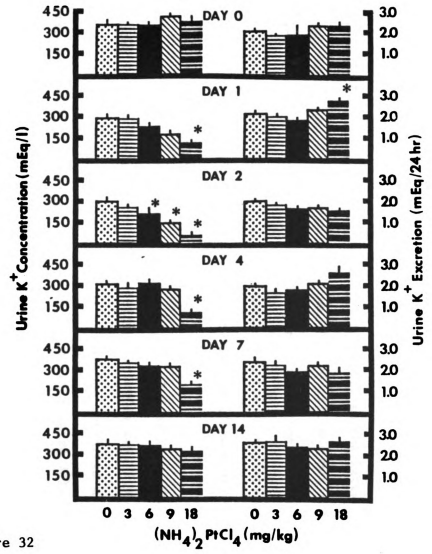

DAY 0
DAY 1
DAY 2
DAY 4
DAY 7
DAY 14
450
300
150
3.0
2.0
1.0
0 3 6 9 18
0 3 6 9 18
(NH4)2PtCl4 (mg/kg)
Urine K+ Concentration (mEq/l)
Urine K+ Excretion (mEq/24 hr)

Figure 32

Treatment with 9 or 18 mg/kg tetrachloroplatinate resulted in decreased urinary sodium (Figure 30) and potassium (Figure 32) concentrations on days 1 and 2 and this effect persisted in the latter group through days 4 and 7. Urinary potassium (Figure 32), but not sodium (Figure 30), excretion was elevated 1 day following treatment with 18 mg/kg tetrachloroplatinate. Smaller doses of tetrachloroplatinate did not affect urinary sodium (Figure 30) or potassium (Figure 32) concentration and excretion.

Blood Urea Nitrogen. Treatment with 7.5 or 15 mg/kg cis-DDP resulted in marked elevations of BUN concentration on days 1 and 2 (Figure 33). Animals treated with 5 mg/kg cis-DDP exhibited a 3-fold increase in BUN on day 4, but not on days 7 or 14 (Figure 33). In contrast, administration of the trans isomer of DDP did not affect BUN concentrations at any time examined (Figure 33). Treatment with 18 mg/kg ammonium tetrachloroplatinate, but not smaller doses, resulted in increased BUN concentrations, an effect observed on days 1 and 2, but not on days 4, 7 or 14 (Figure 33).

Organic Ion Accumulation. The ability of renal cortical slices to accumulate PAH or TEA was not significantly altered in cis-DDP treated animals when compared to pair-fed partners (Figure 34). In contrast, addition of cis-DDP (500 or 600 µg/ml) directly into incubation medium significantly depressed organic ion accumulation by kidney slices (Figure 35). In the presence of 500 or 600 µg/ml cis-DDP, PAH S/M ratios were approximately 48 and 34% of controls, respectively. Similarly, the ability of kidney slices to accumulate TEA in the presence of 500 or 600 µg/ml cis-DDP was 40-50% of controls (Figure 35).

Figure 33. Effect of cis-DDP (top), trans-DDP (center) and ammonium tetrachloroplatinate (bottom) on blood urea nitrogen (BUN) concentrations. Each point with vertical line represents the mean ± SEM of four to five determinations. Identical symbols indicate equimolar platinum treatment. Animals treated with 7.5 or 15 mg/kg cis-DDP did not survive the duration of the 14 day experiment. The stippled area represents the mean ± 95% confidence interval of pair-fed controls. Asterisks and daggers indicate a significant difference from ad-libitum fed and pair-fed controls, respectively, on the corresponding day ($p<0.05$).

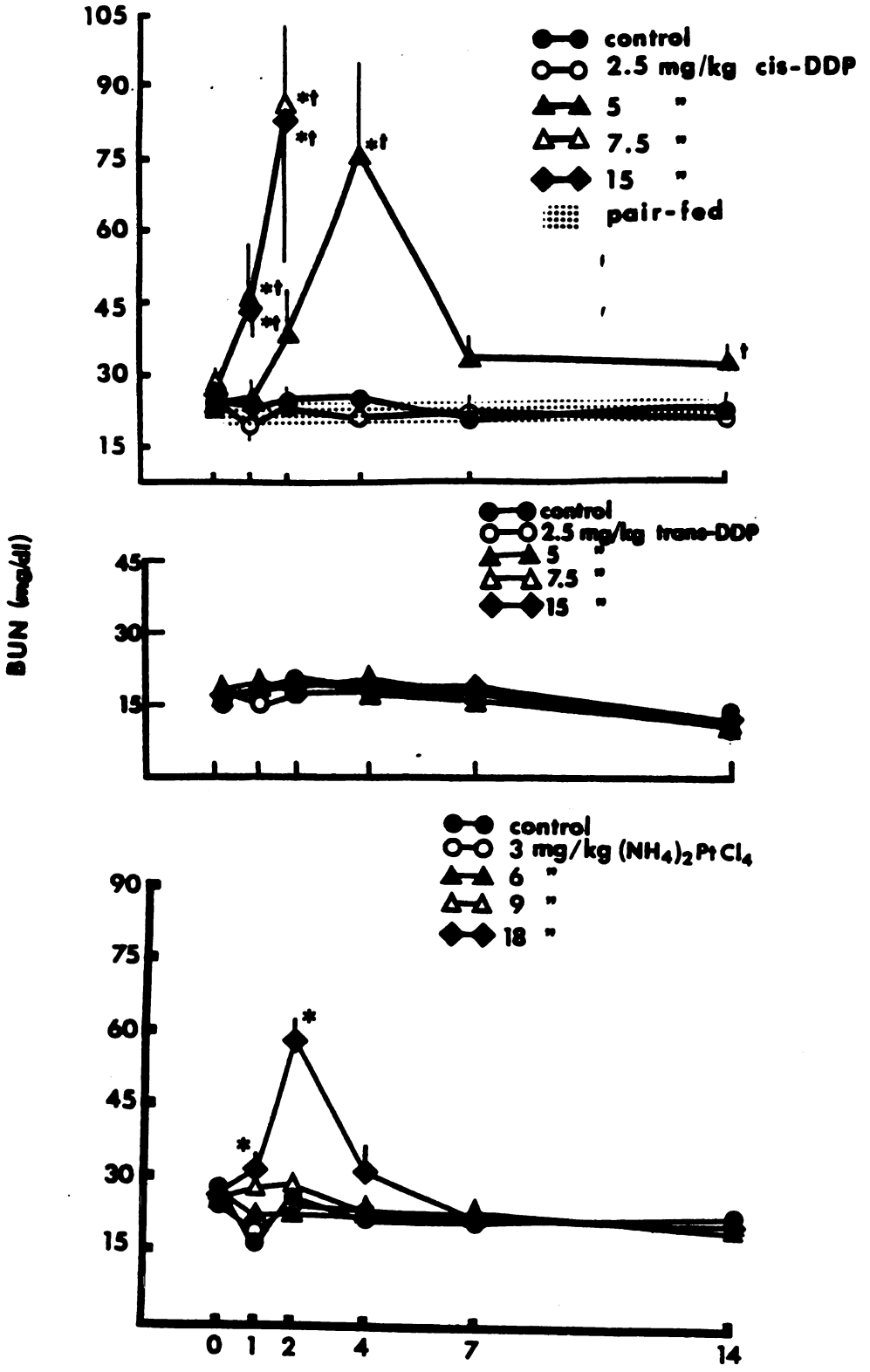

105
90
75
60
45
30
15
control
2.5 mg/kg cis-DDP
5 "
7.5 "
15 "
pair-fed
*†
*†
*†
*†
*†
†
BUN (mg/dl)
45
30
15
control
2.5 mg/kg trans-DDP
5 "
7.5 "
15 "
control
3 mg/kg (NH4)2PtCl4
6 "
9 "
18 "
90
75
60
45
30
15
*
*
0 1 2 4 7 14
Time (days)

Figure 33

Figure 34. PAH and TEA accumulation (slice/medium) (S/M) by renal cortical slices four days following cis-DDP treatment. Each bar represents the mean ± SEM of four determinations. Open bars represent pair-fed partners for each drug treatment.

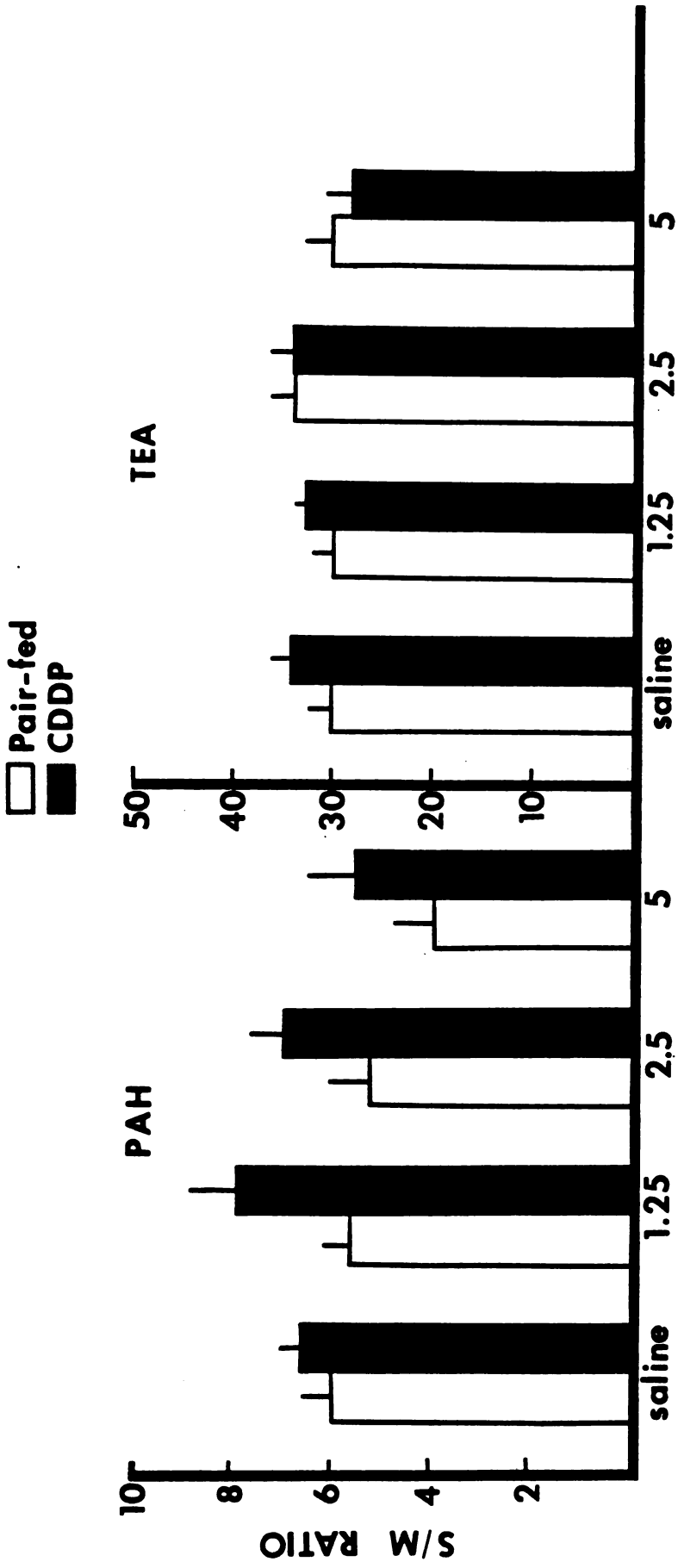
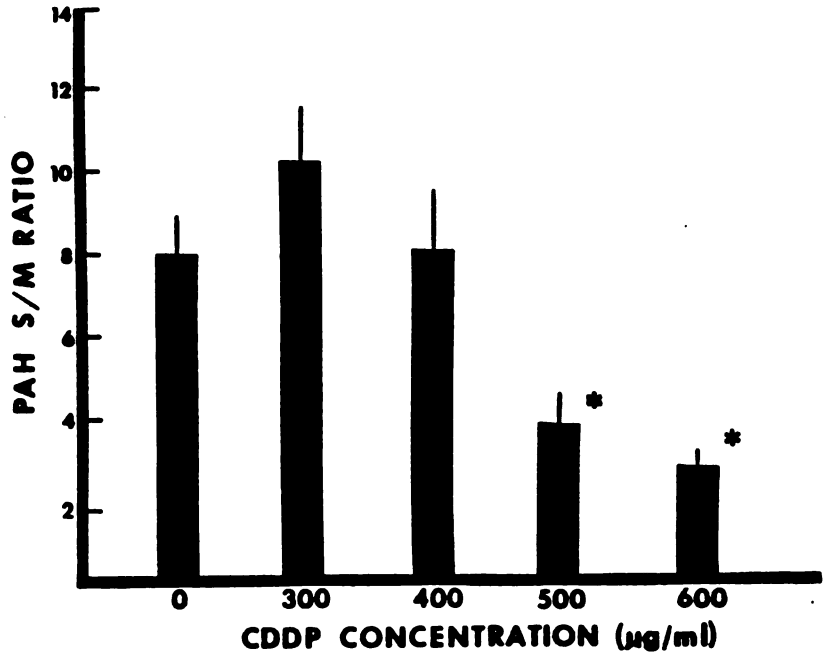

Pair-fed
CDDP
PAH
TEA
S/M RATIO
10
8
6
4
2
50
40
30
20
10
saline
1.25
2.5
5
CDDP (mg/kg)

Figure 34

Figure 35. Effect of addition of cis-DDP to incubation medium on PAH and TEA accumulation (S/M ratio) by renal cortical slices. Each bar represents the mean ± SEM of four experiments. Each experiment consisted of slices pooled from three animals. Asterisks indicate a significant difference from controls ($p<0.01$).


PAH S/M RATIO
14
12
10
8
6
4
2
0
300
400
500
600
CDDP CONCENTRATION (µg/ml)
*
*

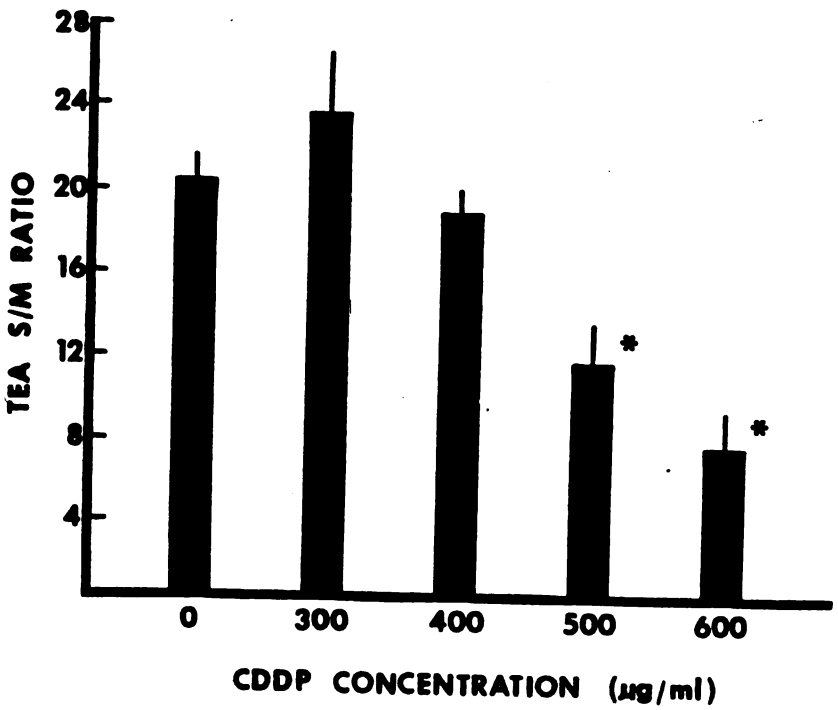

TEA S/M RATIO
28
24
20
16
12
8
4
0
300
400
500
600
CDDP CONCENTRATION (µg/ml)
*
*

Figure 35

Renal Clearances of Inulin and PAH. Both C_{inulin} and C_{PAH} were significantly depressed four days following administration of 5 mg/kg cis-DDP (Table 6) during pre-volume and post-volume expansion periods. Prior to volume expansion, C_{inulin} and C_{PAH} were diminished by approximately 50 and 60%, respectively, in treated animals (Table 6). Following volume expansion, C_{inulin} increased approximately two-fold in both control and treated animals while C_{PAH} increased by 317, 150 and 220% in ad-libitum fed controls, pair-fed controls and cis-DDP treated animals, respectively (Table 6).

Histopathological Effects of cis-DDP on the Kidney. Renal tissue from animals receiving 5 or 7.5 mg/kg cis-DDP exhibited coagulative necrosis of the outer stripe of the outer medulla, primarily affecting the S_3 segment of the proximal tubule (Figures 36 and 37). These proximal tubular cells appeared atrophic with pale vesicular cytoplasm and necrotic foci as indicated by the presence of desquamated epithelial cells and amorphous cellular debris in the tubular lumen (Figures 36 and 37). Treatment with 5 or 7.5 mg/kg cis-DDP also resulted in a slight mononuclear cell infiltration in necrotic regions. There was occasional atrophy of the tubular cells in the cortex. Kidneys of animals treated with 7.5 mg/kg cis-DDP also exhibited dilatation of tubular lumen (Figure 37). Kidneys of animals treated with 2.5 mg/kg cis-DDP did not exhibit significant histological damage (Figures 36 and 37).

TABLE 6

Effects of cis-DDP(5 mg/kg) on Renal Clearance of Inulin (C_{inulin}) and PAH (C_{PAH})

	Pre-Volume Expansion			Post-Volume Expansion		
	Control	cis-DDP	Pair-fed	Control	cis-DDP	Pair-fed
C_{inulin}	1.30±0.21[a]	0.53±0.17[b]	1.25±0.20	2.62±0.23[c]	0.90±0.21[b]	2.59±0.12
C_{PAH}	3.52±0.31	1.35±0.37[b]	3.16±0.60	11.16±1.42	2.03±0.76[b]	6.98±0.74

[a]Values are expressed as means ± SEM of 4-5 experiments. Each experiment consisted of two 20-minute urine collections. Blood (300 µl) was sampled from the femoral artery at the midpoint of each urine collection.

[b]Significantly different from both ad-libitum and pair-fed controls ($p<0.05$).

[c]Values are expressed as ± SEM of 4-5 experiments. Each experiment consisted of infusing a 1:4 rat plasma:saline solution (4% body weight) over a 5-min period. Ten minutes were allowed for equilibration. Blood (300 µl) was sampled at midpoint of one 20-min urine collection.

Figure 36. Renal tissue four days following cis-DDP treatment. Hematoxylin and eosin, 40x. Necrosis of the outer stripe of the outer medulla was evident in kidneys of animals treated with 5 or 7.5 mg/kg cis-DDP. Kidneys of animals treated with 2.5 mg/kg cis-DDP were not markedly different than those of controls.

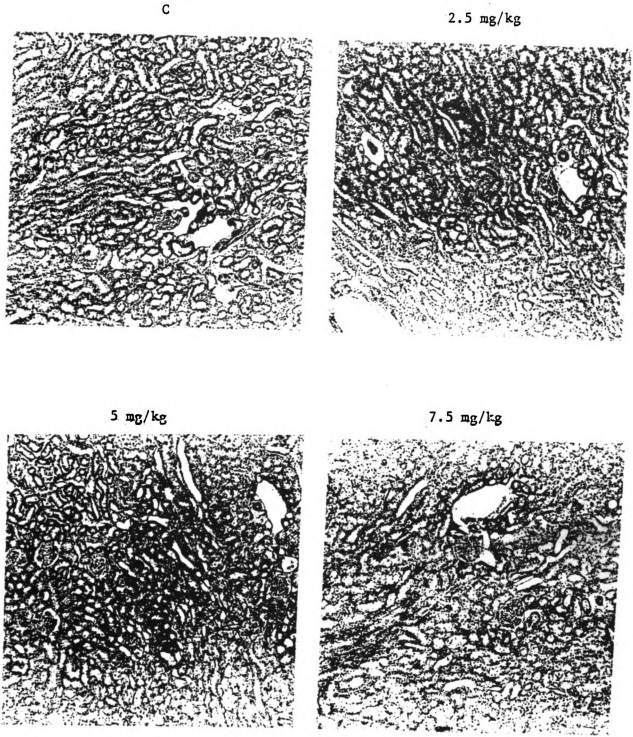


Figure 36

Figure 37. Renal tissue four days following cis-DDP treatment. Hematoxylin and eosin, 100x. Proximal tubular cells of the 5 and 7.5 mg/kg cis-DDP group were atrophic with pale vesicular cytoplasm. Desquamated epithelial cells and amorphous cellular debris filled the tubular lumens. Proximal tubular lumens of kidneys of animals treated with 7.5 mg/kg were markedly dilated. Administration of 2.5 mg/kg cis-DDP did not markedly affect proximal tubules.

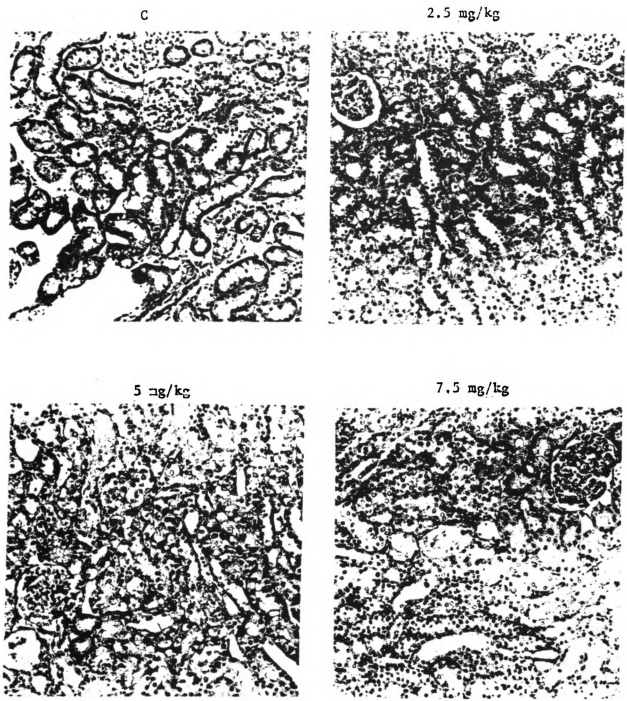


Figure 37

Effect of Mannitol Pretreatment on cis-DDP Glucose Intolerance

Administration of mannitol prior to cis-DDP treatment resulted in a marked reduction in BUN concentration (Table 7). Although mannitol pretreatment did not affect cis-DDP hyperglycemia fifteen minutes following a glucose load, a significant reduction in plasma glucose concentrations was observed at 30, 60 and 120 minutes (Figure 38). Nevertheless, plasma glucose concentrations were elevated in the Mannitol/cis-DDP group compared to controls at 15, 30 and 60 minutes (Figure 38).

Plasma IRI concentrations of cis-DDP treated animals in both the fasting and glucose stimulated state were not significant affected by mannitol pretreatment (Figure 39). Plasma IRG concentrations were significantly elevated in both cis-DDP treated groups (sham and mannitol pretreated) (Figures 39); however, plasma IRG of cis-DDP drug treated animals was significantly reduced by mannitol pretreatment at all time points examined (Figure 39).

Effect of Other Nephrotoxicants on Glucose Tolerance

Blood urea nitrogen and serum creatinine concentrations were significantly elevated following administration of cis-DDP, gentamicin or glycerol (Table 8). Kidney weight to body weight ratios were elevated following treatment with any of the tested nephrotoxicants (Table 8). Glucose tolerance, however, was only impaired following treatment with cis-DDP or glycerol, and not following treatment with cephaloridine or gentamicin (Figure 40). Similarly, the total integrated

TABLE 7

Effect of Mannitol Pretreatment on Blood Urea Nitrogen (BUN) and Plasma Immunoreactive Glucagon (IRG) of cis-DDP Treated Rats

Treatment	BUN (mg/dl)	Plasma IRG (pg/ml)		
		Total[d]	True Pancreatic[e]	Extra-Pancreatic[f]
Control	30± 2[a]	154± 6	74± 8	80±10
Mannitol/ cis-DDP	48± 3[b]	321±33[b,c]	122± 3[b]	199±33[b,c]
Sham/cis-DDP	101±19[c]	835±56[c]	251±43[c]	602±69[c]

[a]Values are expressed as means ± SEM of four determinations. Plasma pooled from 3 identically treated rats was used for one determination. Mannitol pretreatment (2.4 g/kg) involved intravenous infusion of 10% mannitol solution (in 0.45% NaCl) over a 30 minute period using a Harvard infusion pump. Following 25 minutes of mannitol infusion, cis-DDP (5 mg/kg) (Mannitol/cis-DDP) or saline vehicle (controls) was intravenously infused. Mannitol was then infused for remaining 5 minutes. Sham/cis-DDP animals were subjected to similar surgical procedures but did not receive mannitol.

[b]Significantly different from Sham/cis-DDP ($p<0.05$).

[c]Significantly different from controls ($p<0.05$).

[d]Total plasma IRG was determined using an antibody which cross-reacts with all immunoreactive components of glucagon (Leichter et al., 1975).

[e]True pancreatic plasma IRG was determined using Unger's 30K antibody which cross-reacts with 3500 MW form of glucagon.

[f]Extra-pancreatic plasma IRG was calculated as the difference between total and true-pancreatic IRG.

Figure 38. Effect of mannitol pretreatment on cis-DDP induced glucose intolerance. Pretreatment with mannitol (2.4 g/kg) involved intravenous infusion of 10% mannitol over a 30 minute period. After 25 minutes, a bolus injection of cis-DDP (5 mg/kg) (mannitol/cis-DDP) or saline vehicle (control) was infused intravenously and mannitol infused for the remaining five minutes. Sham/cis-DDP animals were subjected to similar surgical procedures with the exception that they received cis-DDP alone, without mannitol infusion. Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks indicate a significant difference from controls at the corresponding time point. Daggers indicate a significant difference from mannitol/cis-DDP animals at the corresponding time point ($p<0.05$).

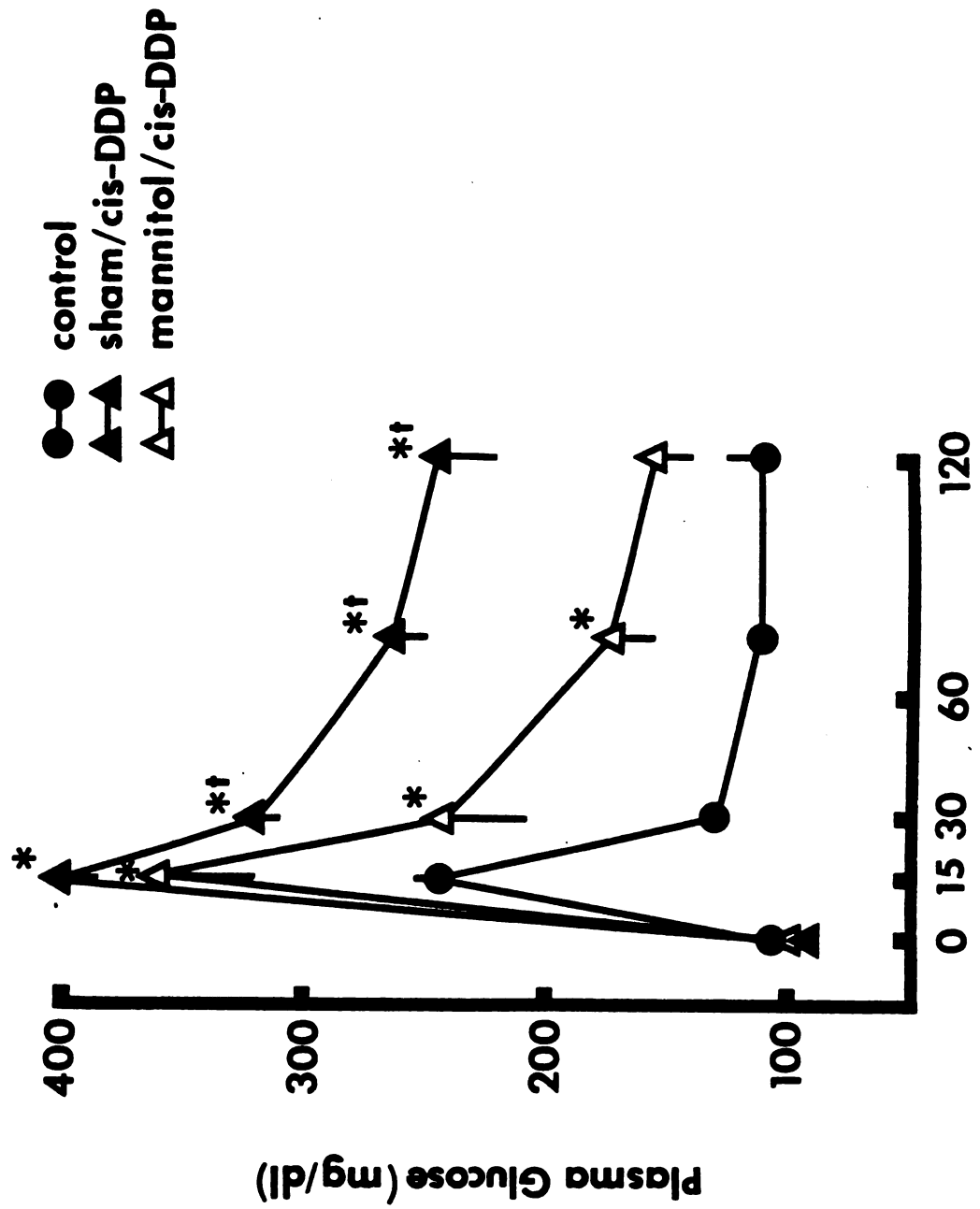

control
sham/cis-DDP
mannitol/cis-DDP
Plasma Glucose (mg/dl)
400
300
200
100
0
15
30
60
120
Time (min)

Figure 38

Figure 39. Effects of mannitol pretreatment on plasma immunoreactive insulin (IRI) and glucagon (IRG) concentrations of cis-DDP treated animals. Pretreatment with mannitol (2.4 g/kg) involved intravenous infusion of 10% mannitol over a 30 minute period. After 25 minutes, a bolus injection of cis-DDP (5 mg/kg) (mannitol/cis-DDP) or saline vehicle (control) was infused intravenously and mannitol infused for the remaining five minutes. Sham/cis-DDP animals were subjected to similar surgical procedures with the exception that they received cis-DDP alone, without mannitol infusion. Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks indicate a significant difference from controls at the corresponding time point. Daggers indicate a significant difference from mannitol/cis-DDP animals at the corresponding time point ($p<0.05$).

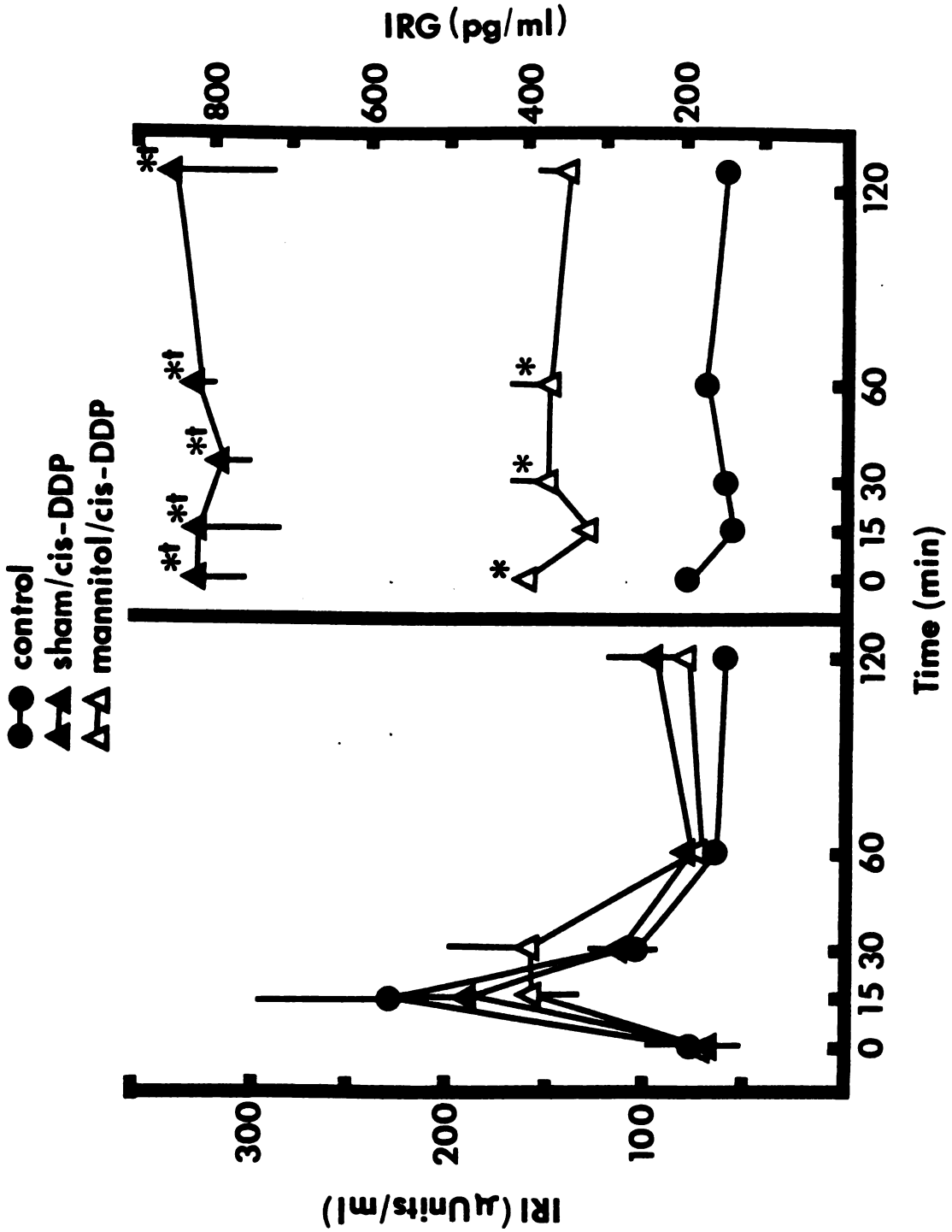

control
sham/cis-DDP
mannitol/cis-DDP
IRI (μUnits/ml)
IRG (pg/ml)
Time (min)
0 15 30 60 120
100 200 300
200 400 600 800

Figure 39

Figure 39. Effects of mannitol pretreatment on plasma immunoreactive insulin (IRI) and glucagon (IRG) concentrations of cis-DDP treated animals. Pretreatment with mannitol (2.4 g/kg) involved intravenous infusion of 10% mannitol over a 30 minute period. After 25 minutes, a bolus injection of cis-DDP (5 mg/kg) (mannitol/cis-DDP) or saline vehicle (control) was infused intravenously and mannitol infused for the remaining five minutes. Sham/cis-DDP animals were subjected to similar surgical procedures with the exception that they received cis-DDP alone, without mannitol infusion. Each point with vertical line represents the mean ± SEM of four determinations. Plasma pooled from three identically treated animals was used for one determination. Asterisks indicate a significant difference from controls at the corresponding time point. Daggers indicate a significant difference from mannitol/cis-DDP animals at the corresponding time point ($p<0.05$).

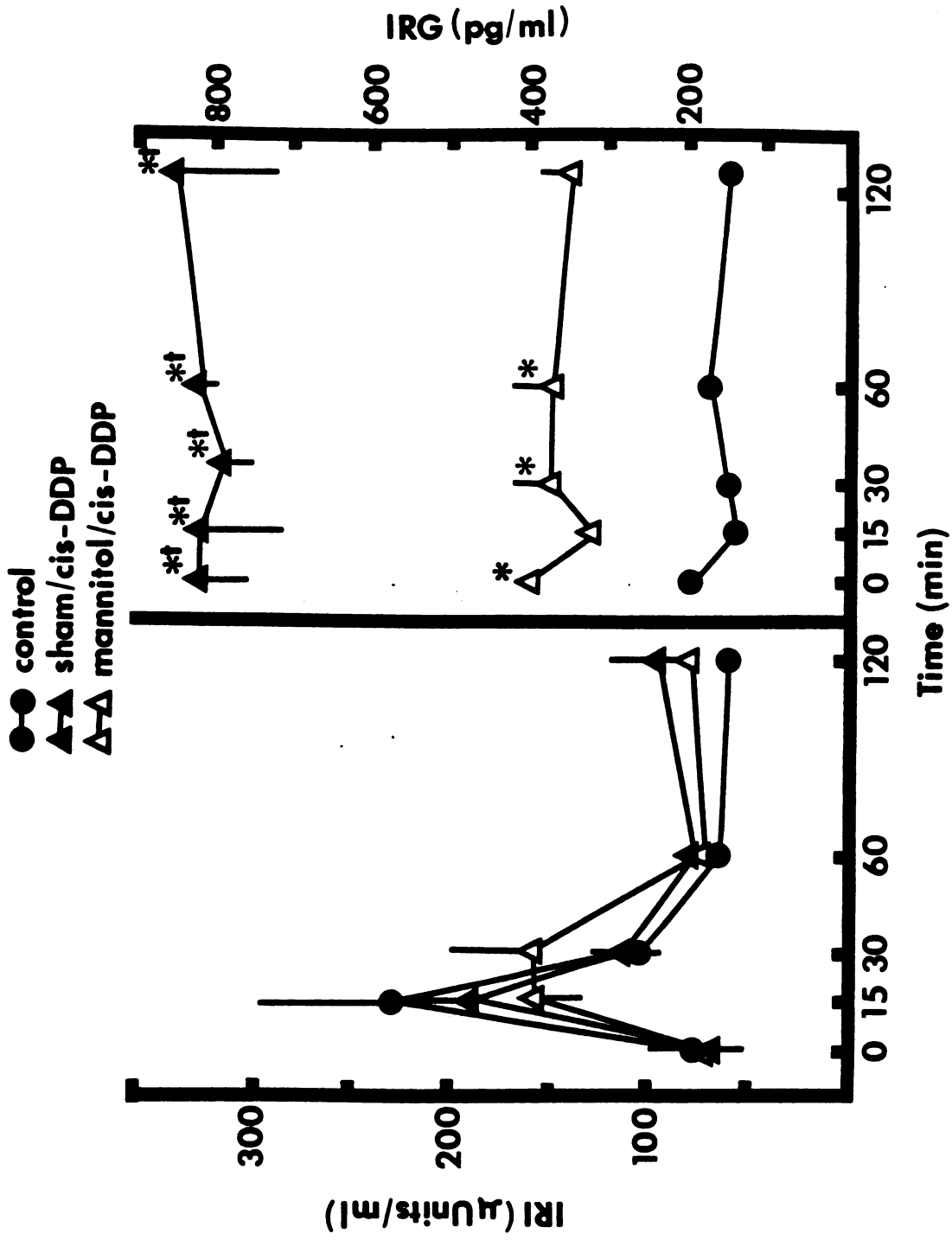

control
sham/cis-DDP
mannitol/cis-DDP
IRI (μUnits/ml)
IRG (pg/ml)
Time (min)
0 15 30 60 120
100
200
300
200
400
600
800
*
*t

Figure 39

TABLE 8

Effect of Selected Nephrotoxicants on Renal Function and Glucose Tolerance

Treatment	Time After Treatment (days)	$\frac{\text{Kidney Weight (g)}}{\text{Body Weight (g)}} \times 10^2$	Blood Urea Nitrogen (mg/dl)	Serum Creatinine (mg/dl)	Total Glycemic Response[a] (g/dl x minutes)
Control	4	0.74±0.02[b]	35± 3	1.2±0.1	24.0±1.4
Cephaloridine (1000 mg/kg, ip)	4	1.03±0.08[c]	50±10	1.4±0.1	23.4±3.0
cis-DDP (5 mg/kg, iv)	4	0.83±0.02[c]	65± 2[c]	2.1±0.1[c]	60.4±7.6[c]
Gentamicin (30 mg/kg, ip x 2x/da x 8 days)	9	0.98±0.01[c]	124± 8[c]	3.6±0.4[c]	21.6±3.3
Glycerol (10 ml/kg, im of 50% w/v)	1	0.99±0.03[c]	170±10[c]	4.1±0.4[c]	64.1±5.4[c]

[a]Values represent area under plasma glucose curve 15, 30, 60 and 120 minutes following glucose load (2 g/kg, i.p.). Area was calculated using the following formula: Area = ½(15a + 45b + 90c + 180d + 120e) - 120a, where a, b, c, d, and e represent plasma glucose concentration at 0, 15, 30, 60 and 120 minutes, respectively.

[b]Values are expressed as means ± SEM of 3-4 determinations.

[c]Significantly different from controls ($p<0.05$).

Figure 40. Effect of selected nephrotoxicants on glucose tolerance. Treatments included cephaloridine (1000 mg/kg, ip), cis-DDP (5 mg/kg, iv) gentamicin (30 mg/kg, ip x 2x/day x 8 days), glycerol (10 ml/kg, im of 50% w/v) or a saline vehicle. Glucose tolerance was evaluated 4 days following cephaloridine or cis-DDP, the 9th day of gentamicin treatment and 1 day following glycerol. Plasma glucose concentration is plotted against time following a glucose load (2 g/kg, ip). Each point with vertical line represents the mean ± SEM of three to four determinations. Asterisks indicate a significant difference from controls at the corresponding time point ($p<0.05$).

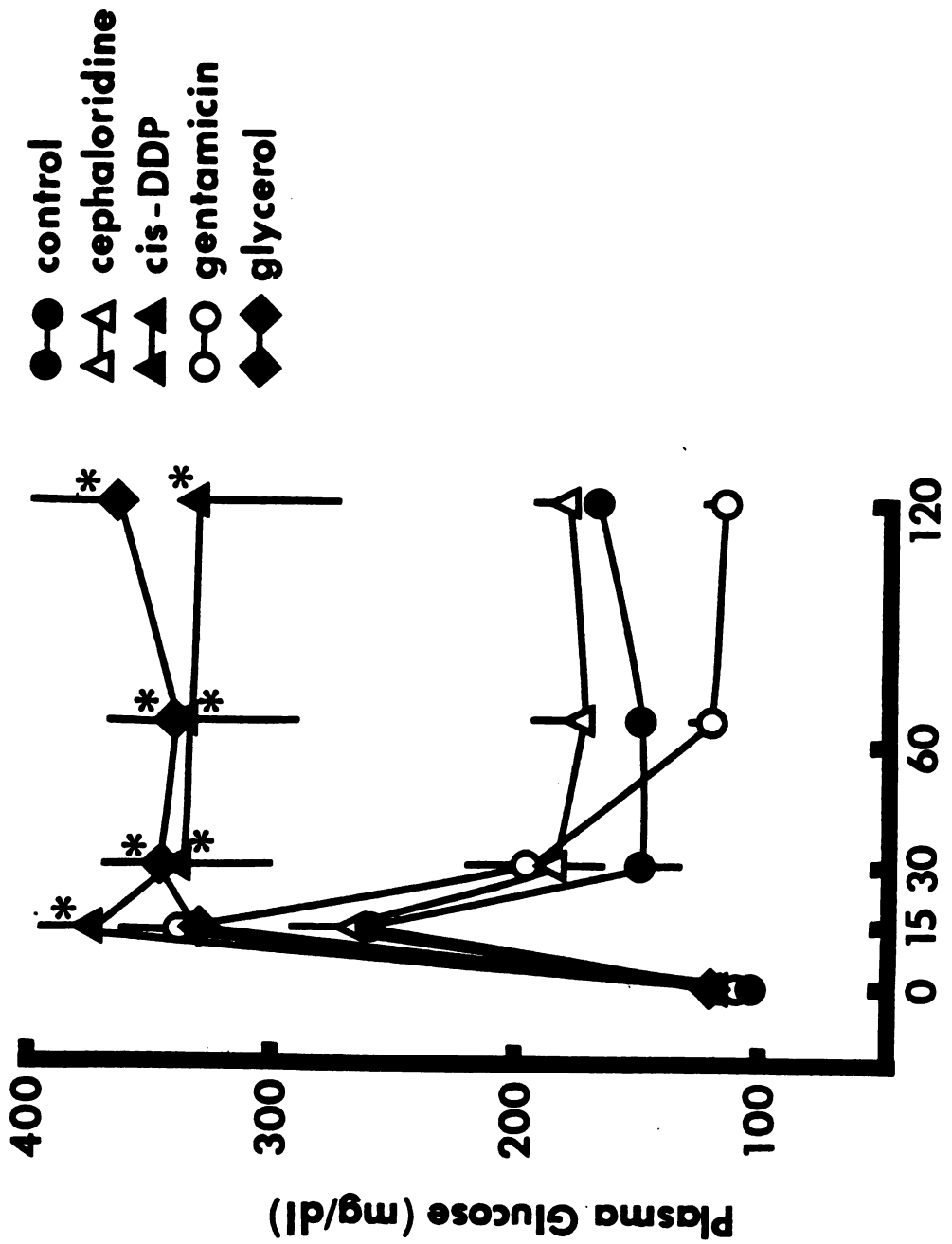

control
cephaloridine
cis-DDP
gentamicin
glycerol
Plasma Glucose (mg/dl)
400
300
200
100
0
15
30
60
120
Time (min)

Figure 40

glycemic response to glucose administration was elevated only in animals treated with cis-DDP or glycerol (Table 8).

Effect of cis-DDP on Pancreatic Structure and Function

Pancreatic tissue from cis-DDP treated rats (2.5, 5 or 7.5 mg/kg) appeared similar to that of controls (Figure 41). No signs of islet degeneration, necrosis, or inflammation were apparent at any of the drug doses tested (Figure 41). cis-DDP had no effect on serum amylase activity (Figure 42).

Biochemical Correlates of cis-DDP Glucose Intolerance

Serum Sodium, Potassium, Calcium and Phosphorus

cis-DDP treatment (2.5, 5, or 7.5 mg/kg) did not affect serum sodium, potassium or calcium concentrations on day 4 (Table 9). However, administration of 7.5 mg/kg cis-DDP resulted in serum phosphorus concentrations approximately two times greater than controls (Table 9).

Fasting Plasma Glucose and Hepatic and Renal Gluconeogenic Enzyme Activity

Fasting plasma glucose concentrations of animals treated with 2.5 or 5 mg/kg cis-DDP were comparable to controls (Table 10). In contrast, fasting plasma glucose was markedly elevated four days following administration of 7.5 mg/kg cis-DDP (Table 10). cis-DDP treatment (2.5, 5 or 7.5 mg/kg) had no effect on hepatic or renal G-6-Pase or FDPase activities (Table 10) on day 4.

Figure 41. Pancreatic tissue from rats treated with cis-DDP. Hematoxylin and eosin stain, x100. Lighter stained tissue represents pancreatic islets. No gross differences in histological appearance of pancreatic tissue are detectable following treatment with 2.5, 5 or 7.5 mg/kg cis-DDP.

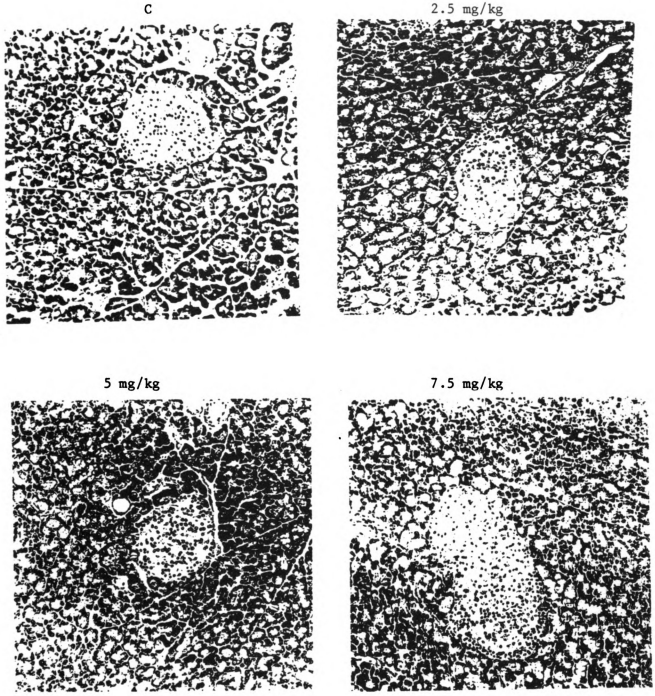


Figure 41

Figure 42. Serum amylase activity four days following cis-DDP treatment. Each bar with vertical line represents the mean ± SEM of four determinations. Serum pooled from three identically treated animals was used for one determination. Pair-fed animals were offered the measured amount of food consumed by the 5 mg/kg group.

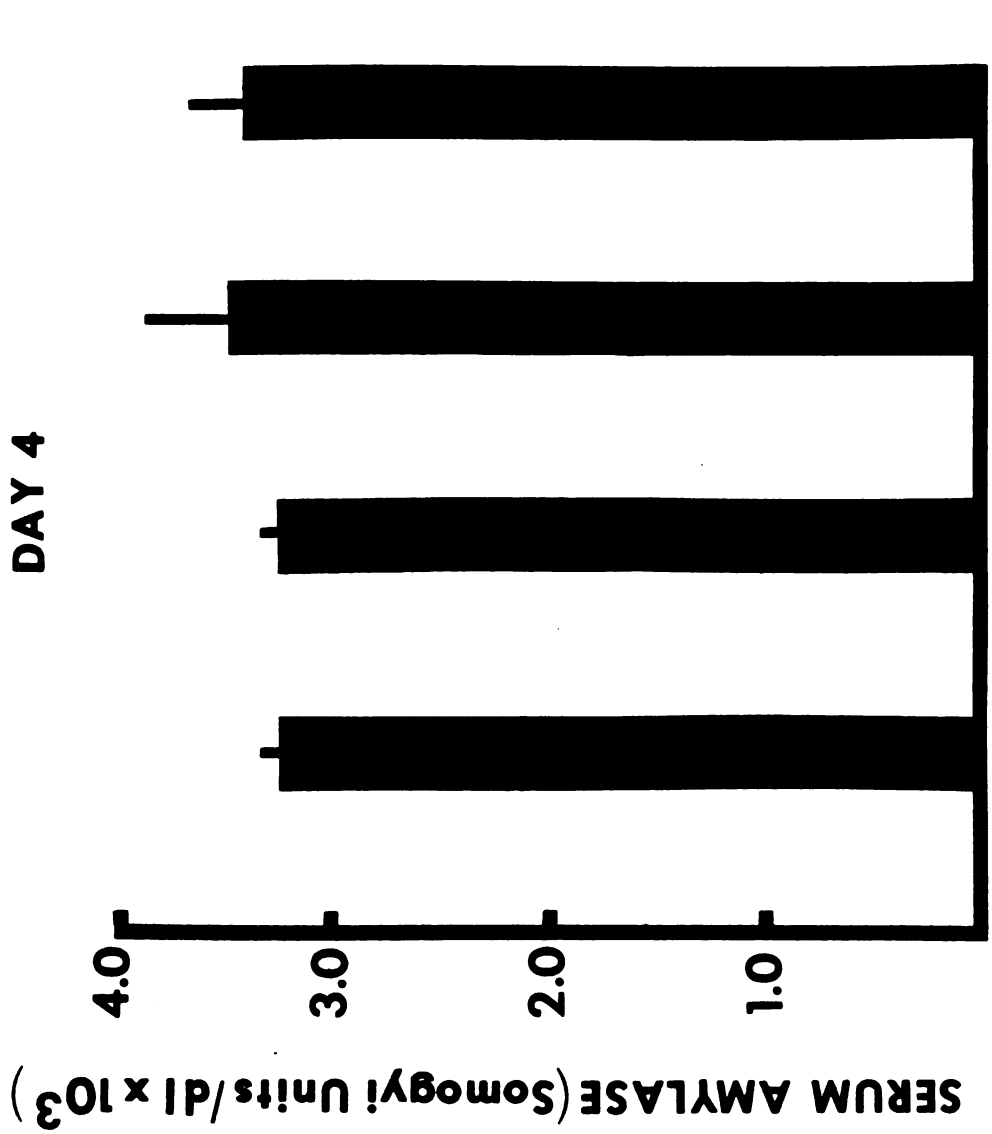

DAY 4
SERUM AMYLASE (Somogyi Units/dl x 10³)
4.0
3.0
2.0
1.0
control
2.5
5
pair-fed
cis-DDP (mg/kg)

Figure 42

TABLE 9

Effect of cis-DDP on Serum Sodium, Potassium, Calcium and Phosphorus

cis-DDP (mg/kg)	Sodium (mEq/l)	Potassium (mEq/l)	Calcium (mg/dl)	Phosphorus (mg/dl)
0	139±4[b]	4.8±0.3	9.5±0.4	6.2±0.2
2.5	137±4	4.2±0.1	10.2±0.2	5.9±0.1
5	138±2	4.1±0.2	9.0±0.3	5.8±0.2
7.5	138±1	4.5±0.2	9.1±0.2	11.2±1.7[c]
Pair-fed[a]	144±4	4.9±0.4	9.1±0.3	6.0±0.3

[a]Pair-fed animals were offered the measured amount of food consumed by the 7.5 mg/kg group.

[b]Values are expressed as means ± SEM of 4-5 determinations. Experiments were conducted 4 days following cis-DDP or vehicle administration. Animals were fasted 4-5 hours prior to experimentation.

[c]Significantly different from both ad-libitum and pair-fed controls ($p<0.05$).

TABLE 10

Selected Biochemical and Endocrine Measurements of Glucose Metabolism Following cis-DDP Treatment

cis-DDP (mg/kg)	Glucose-6-Phosphatase (μmoles phosphate/hr/mg protein)		Fructose-1,6-Diphosphatase (μmoles phosphate/hr/mg protein)		Plasma Glucose (mg/dl)	Plasma IRI (μUnits/ml)	Plasma IRG (pg/ml)
	Hepatic	Renal	Hepatic	Renal			
0	9.81±0.83[a]	8.32±0.64	1.94±0.48	2.60±0.20	98± 4	40±5	179± 7
2.5	11.23±1.26	7.70±1.08	1.77±0.22	2.57±0.19	89± 4	32±4	188± 8
5	11.61±1.84	6.13±0.31	1.58±0.14	2.20±0.50	97± 7	27±6	344±70[c]
7.5	8.19±1.00	8.57±0.50	1.92±0.17	2.09±0.38	132±10[c]	60±7[c]	>1600[d]
Pair-fed[b]	9.93±1.10	9.50±1.40	1.56±0.15	2.41±0.16	97± 7	25±4	184± 5

[a]Values are expressed as means ± SEM of at least 4 determinations. Experiments were conducted 4 days following cis-DDP or vehicle administration. All animals were fasted overnight (16 hours) prior to experimentation.

[b]Pair-fed animals were offered the measured amount of food consumed by the 7.5 mg/kg group.

[c]Significantly different from both ad-libitum and pair-fed controls ($p<0.05$).

[d]All values were >1600 pg/ml and beyond limits of assay sensitivity. Sample volumes were insufficient to repeat assay.

Endocrine Correlates of cis-DDP Glucose Intolerance

IRI and IRG Concentrations in Plasma and Pancreas

Although fasting plasma IRI concentrations of animals treated with 2.5 or 5 mg/kg cis-DDP were comparable to controls, those treated with 7.5 mg/kg cis-DDP were significantly elevated compared to ad-libitum and pair-fed controls (Table 10). Fasting hyperglucagonemia was apparent four days following treatment with either 5 or 7.5 mg/kg cis-DDP.

cis-DDP treatment at doses of 2.5, 5 or 7.5 mg/kg, did not significantly affect pancreatic concentrations of IRI (Figure 43) or IRG (Figure 44) in either the head portion (duodenal lobe) or body and tail region (adjacent to spleen and stomach).

Components of Plasma IRG

Treatment with 5 or 7.5 mg/kg cis-DDP resulted in a profound increase in total circulating concentrations of IRG on day 4 (Table 11). When an antibody specific to the 3500 MW form of IRG ("true pancreatic" glucagon) was used, a significant elevation in plasma IRG was also observed in the 7.5 mg/kg group when compared to ad-libitum fed controls (Table 11). An elevation in "true pancreatic" plasma IRG was also apparent in the 5 mg/kg cis-DDP group when compared to pair-fed partners (Table 11). A marked elevation in "extra-pancreatic" plasma IRG (total - "true" IRG) was evident in these latter two treatment groups (Table 11). Plasma IRG ("total", "true pancreatic", and "extra-pancreatic") in cis-DDP (5 mg/kg) treated animals was significantly reduced by mannitol pretreatment (Table 7). Nevertheless, total and "extra-pancreatic" plasma IRG of Mannitol/cis-DDP treated animals was elevated

Figure 43. Pancreatic concentrations of immunoreactive insulin (IRI) four days following cis-DDP treatment. Each bar with vertical line represents the mean ± SEM of four to five determinations. Pancreatic IRI concentration was determined in the head portion of the pancreas (adjacent to duodenum) and the body and tail (adjacent to stomach and spleen). Pair-fed animals were offered the measured amount of food consumed by the 7.5 mg/kg group.

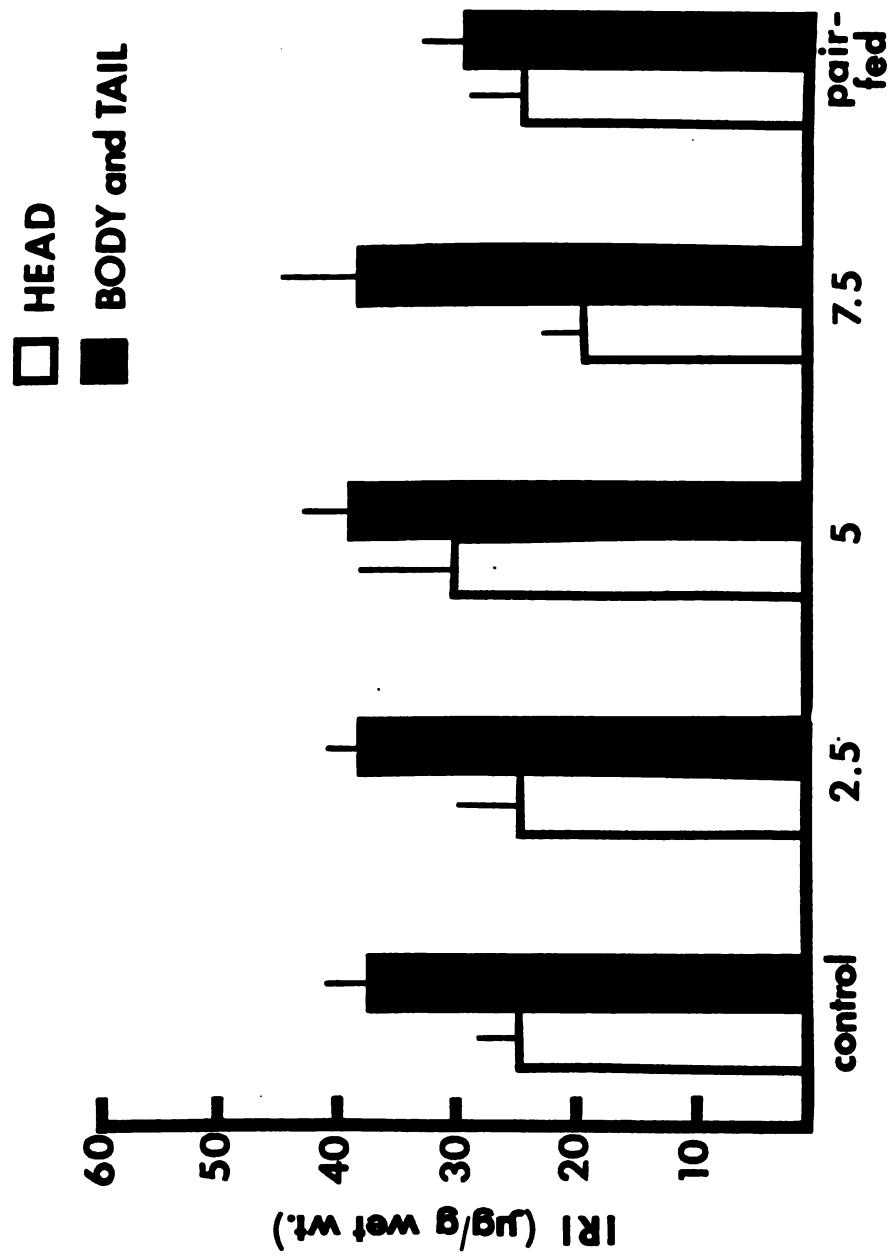

HEAD
BODY and TAIL
IRI (µg/g wet wt.)
60
50
40
30
20
10
control
2.5
5
7.5
pair-fed
cis-DDP (mg/kg)

Figure 43

Figure 44. Pancreatic concentrations of immunoreactive glucagon (IRG) four days following cis-DDP treatment. Each bar with vertical line represents the mean ± SEM of four to five determinations. Pancreatic IRG concentration was determined in the head portion of the pancreas (adjacent to duodenum) and the body and tail (adjacent to stomach and spleen). Pair-fed animals were offered the measured amount of food consumed by the 7.5 mg/kg group.

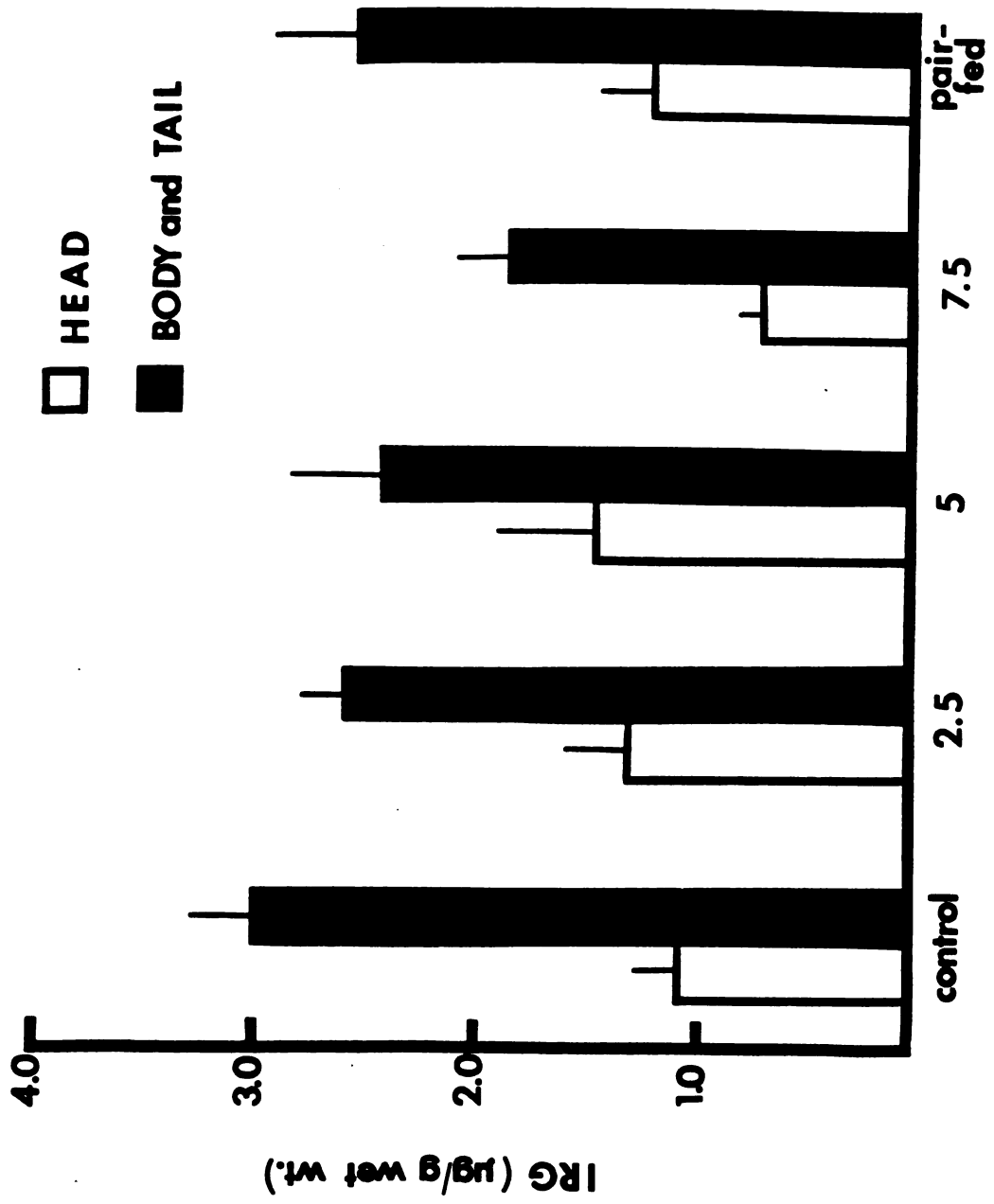

HEAD
BODY and TAIL
4.0
3.0
2.0
1.0
IRG (µg/g wet wt.)
control
2.5
5
7.5
pair-fed
cis-DDP (mg/kg)

Figure 44

Figure 44. Pancreatic concentrations of immunoreactive glucagon (IRG) four days following cis-DDP treatment. Each bar with vertical line represents the mean ± SEM of four to five determinations. Pancreatic IRG concentration was determined in the head portion of the pancreas (adjacent to duodenum) and the body and tail (adjacent to stomach and spleen). Pair-fed animals were offered the measured amount of food consumed by the 7.5 mg/kg group.

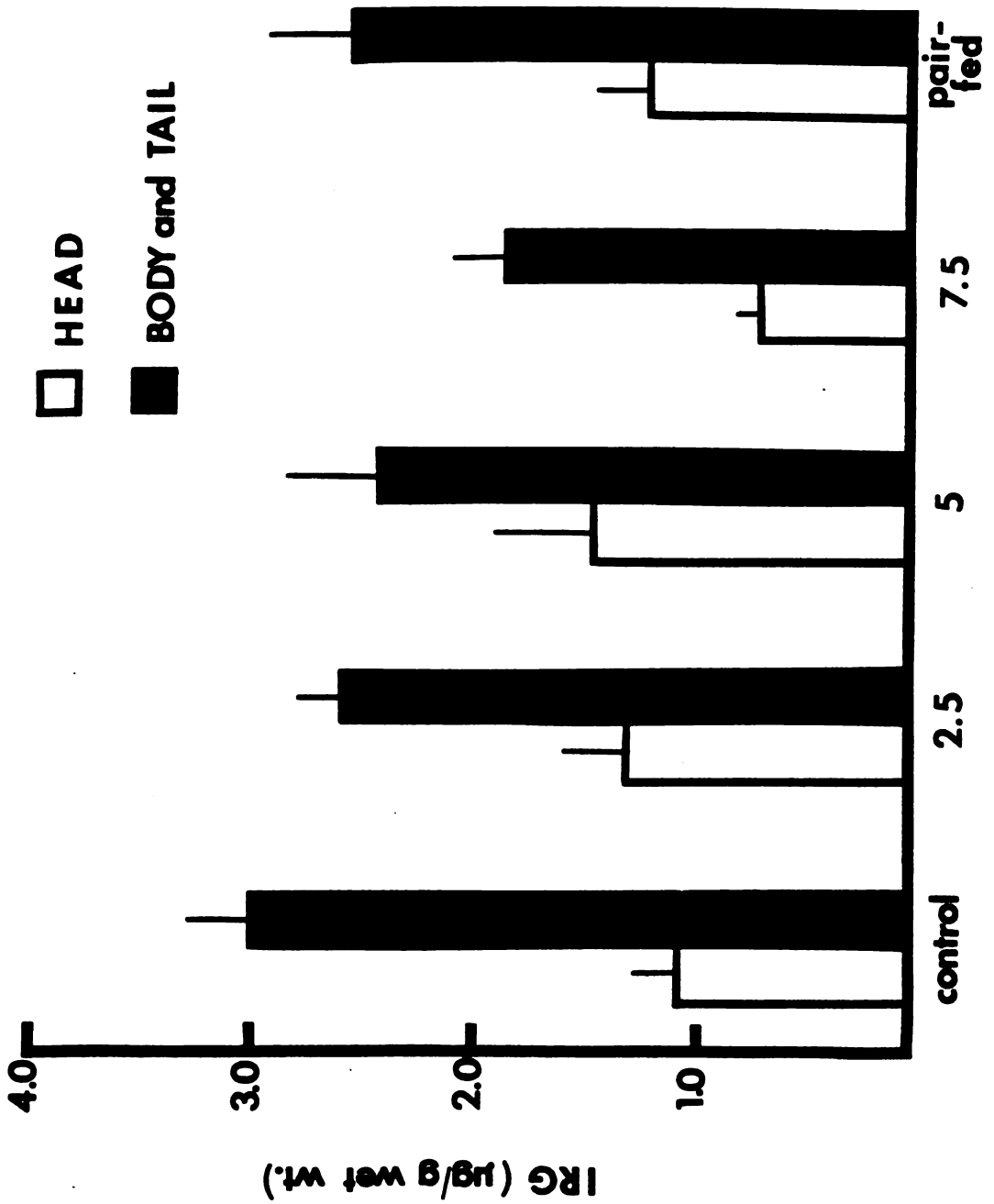

HEAD
BODY and TAIL
4.0
3.0
2.0
1.0
IRG (µg/g wet wt.)
control
2.5
5
7.5
pair-fed
cis-DDP (mg/kg)

Figure 44

TABLE 11

Components of Plasma Immunoreactive Glucagon (IRG)
Following cis-DDP Treatment

cis-DDP (mg/kg)	Plasma IRG (log)		
	Total[a]	True-Pancreatic[b]	Extra-Pancreatic[c]
0	2.32±0.04[d]	2.04±0.04	0.26±0.03
2.5	2.34±0.06	2.04±0.02	0.31±0.06
5	2.61±0.03[e,f]	2.10±0.01[f]	0.51±0.02[e,f]
7.5	3.56±0.06[e,f]	2.73±0.05[e,f]	0.79±0.02[e,f]
Pair-fed	2.15±0.02[e]	1.94±0.05[e]	0.21±0.03[e]

[a]Total plasma IRG was determined using an antibody which cross-reacts with all immunoreactive components of glucagon (Leichter et al., 1975).

[b]True pancreatic IRG was determined using Unger's 30K antibody which cross-reacts with 3500 MW form of glucagon.

[c]Extra-pancreatic IRG was calculated as difference between total and true pancreatic IRG.

[d]Values are expressed as means ± SEM of five determinations. Experiments were conducted four days following drug or vehicle administration. Pair-fed animals were offered the measured amount of food consumed by the 7.5 mg/kg group.

[e]Significantly different from ad-libitum fed controls ($p<0.05$).

[f]Significantly different from pair-fed controls ($p<0.05$).

compared to controls (Table 7). "Total", "true pancreatic" and "extra-pancreatic" plasma IRG was also elevated following treatment with gentamicin and glycerol (Table 12).

Glucagon Sensitivity

Ten minutes following exogenous glucagon administration, plasma glucose concentrations were similar between control and treated (2.5, 5 or 7.5 mg/kg) animals (Table 13). However, a significant elevation in plasma glucose was observed 20 and 30, but not 60, minutes following glucagon administration in the 7.5 mg/kg cis-DDP group. No differences were noted between controls and animals treated with smaller doses of the drug (Table 13). The total integrated glycemic response to exogenous glucagon was elevated only in the 7.5 mg/kg group (Figure 45).

Insulin Sensitivity

Ten, fifteen and twenty minutes following exogenous insulin administration, a marked elevation in plasma glucose was evident in the 5 mg/kg group compared to controls (Figure 46). However, plasma glucose concentrations of treated animals declined to similar levels as controls 30 and 60 minutes following insulin administration (Figure 46).

TABLE 12

Effect of Selected Nephrotoxicants on Plasma Immunoreactive Glucagon (IRG)

Treatment	Time After Treatment	Plasma IRG (pg/ml)		
		Total[a]	True Pancreatic[b]	Extra Pancreatic[c]
Control	4	287± 29[d]	125± 7	162± 34
Cephaloridine (1000 mg/kg, ip)	4	377± 30	154± 8	223± 37
cis-DDP (5 mg/kg, iv)	4	917±197[e]	359±140[e]	558±114[e]
Gentamicin (30 mg/kg, ip x 2x/day x 8 days)	9	1155±153[e]	625±185[e]	529±214[e]
Glycerol (10 ml/kg, im of 50% w/v)	1	967±124[e]	1082±146[e]	

[a]Total plasma IRG was determined using an antibody which cross-reacts with all immunoreactive components of glucagon (Leichter et al., 1975).

[b]True pancreatic plasma IRG was determined using Unger's 30K antibody which cross-reacts with 3500 MW form of glucagon.

[c]Extrapancreatic plasma IRG was calculated as the difference between total and true pancreatic plasma.

[d]Values are expressed as means ± SEM of 3-4 determinations.

[e]Significantly different from controls ($p<0.05$).

TABLE 13

Effect of cis-DDP on Glycemic Response to Exogenous Glucagon

cis-DDP (mg/kg)	Plasma Glucose (mg/dl)				
	Time (minutes) Following Glucagon (1 mg/kg, i.p.)				
	0	10	20	30	60
0	113± 4[a]	182±13	155±15	159±11	176±14
2.5	131±14	173±17	153±17	151±18	168±22
5	104±12	175±30	175±10	177±14	199±25
7.5	122±15	221±68	229±57[b]	243±80[b]	254±98
Pair-fed	82± 7	122± 9	107± 2	106± 7	129±17

[a]Values are expressed as means ± SEM of four determinations. Experiments were conducted 4 days following drug or vehicle administration. Pair-fed animals were offered the measured amount of food consumed by the 7.5 mg/kg group.

[b]Significantly different from pair-fed controls ($p<0.05$).

Figure 45. Area under the glucose curve following exogenous glucagon (1 mg/kg, ip) administration in cis-DDP treated animals. Area was calculated using the following formula: area = $\frac{1}{2}$(10a + 30b + 50c + 90d + 60e) - 60a where a, b, c, d, and e represents plasma glucose concentration at 0, 10, 20, 30 and 60 minutes following glucagon administration. Experiments were conducted four days following cis-DDP or vehicle administration. Pair-fed animals were offered the measured amount of food consumed by the 7.5 mg/kg group. Each bar with vertical line represents the mean ± SEM of four determinations. Asterisks and daggers indicate a significant difference from ad-libitum and pair-fed controls, respectively ($p<0.05$).

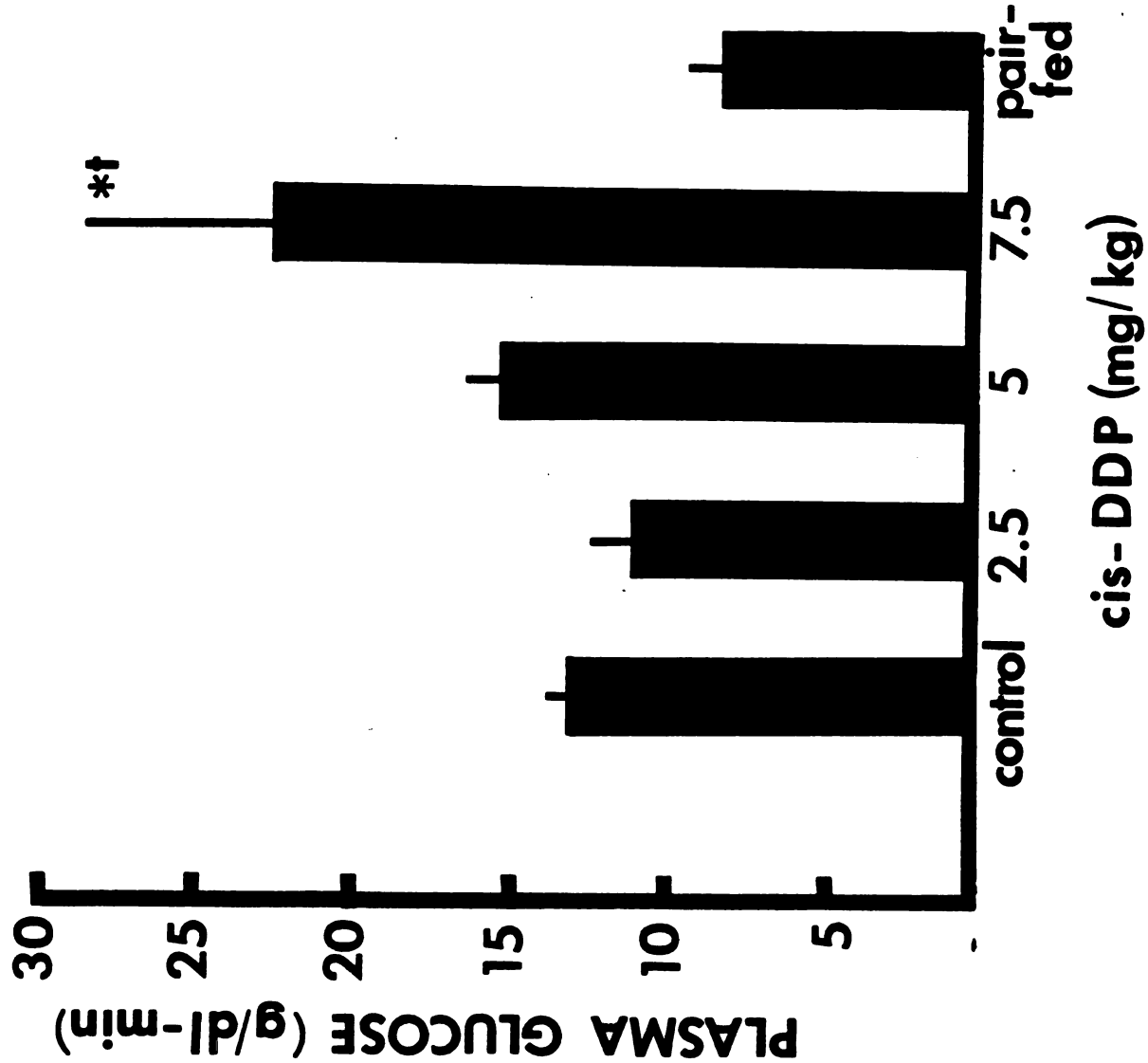

PLASMA GLUCOSE (g/dl-min)
30
25
20
15
10
5
*†
control
2.5
5
7.5
pair-fed
cis-DDP (mg/kg)

Figure 45

Figure 45. Area under the glucose curve following exogenous glucagon (1 mg/kg, ip) administration in cis-DDP treated animals. Area was calculated using the following formula: area = $\frac{1}{2}$(10a + 30b + 50c + 90d + 60e) - 60a where a, b, c, d, and e represents plasma glucose concentration at 0, 10, 20, 30 and 60 minutes following glucagon administration. Experiments were conducted four days following cis-DDP or vehicle administration. Pair-fed animals were offered the measured amount of food consumed by the 7.5 mg/kg group. Each bar with vertical line represents the mean ± SEM of four determinations. Asterisks and daggers indicate a significant difference from ad-libitum and pair-fed controls, respectively ($p<0.05$).

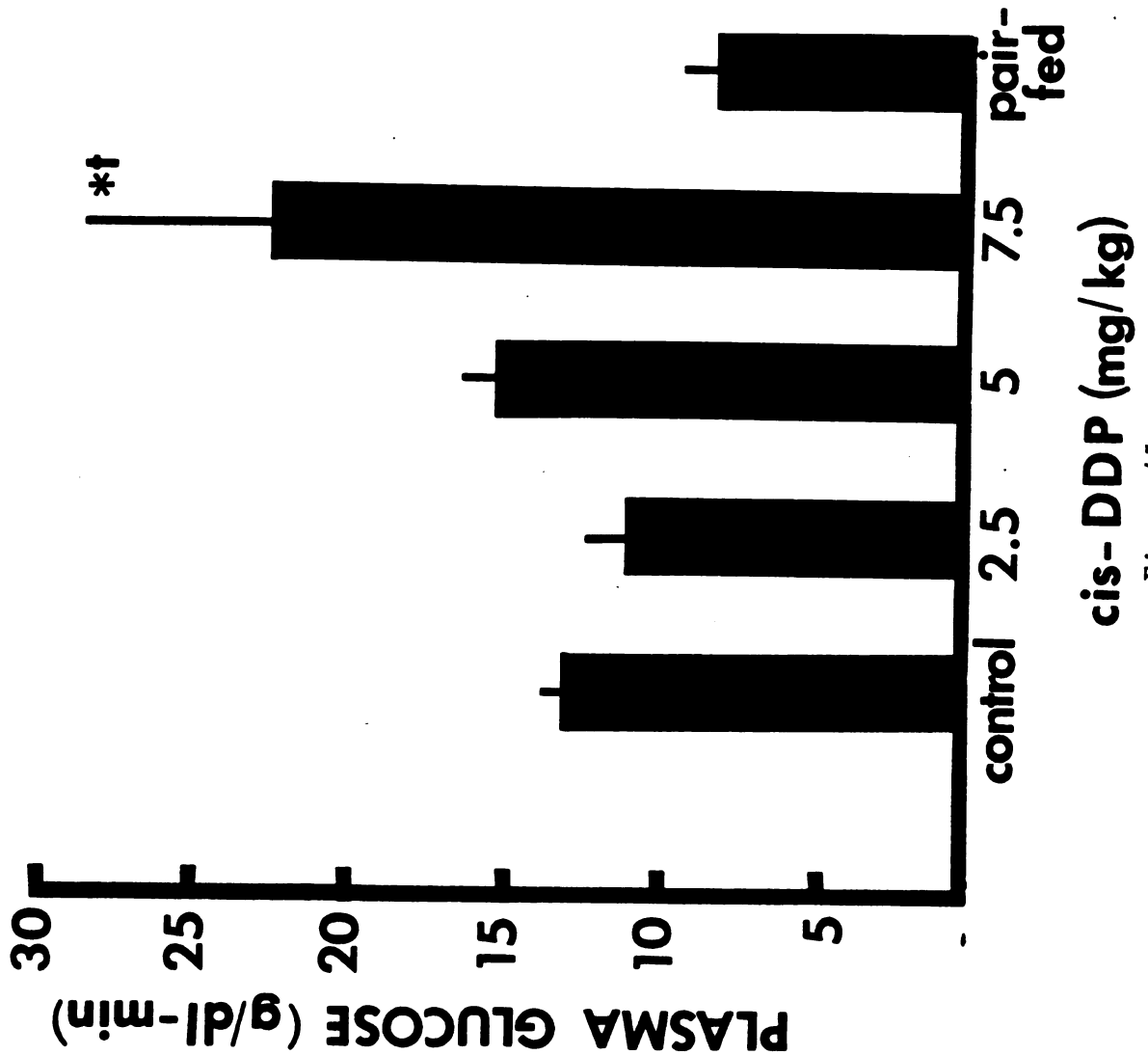
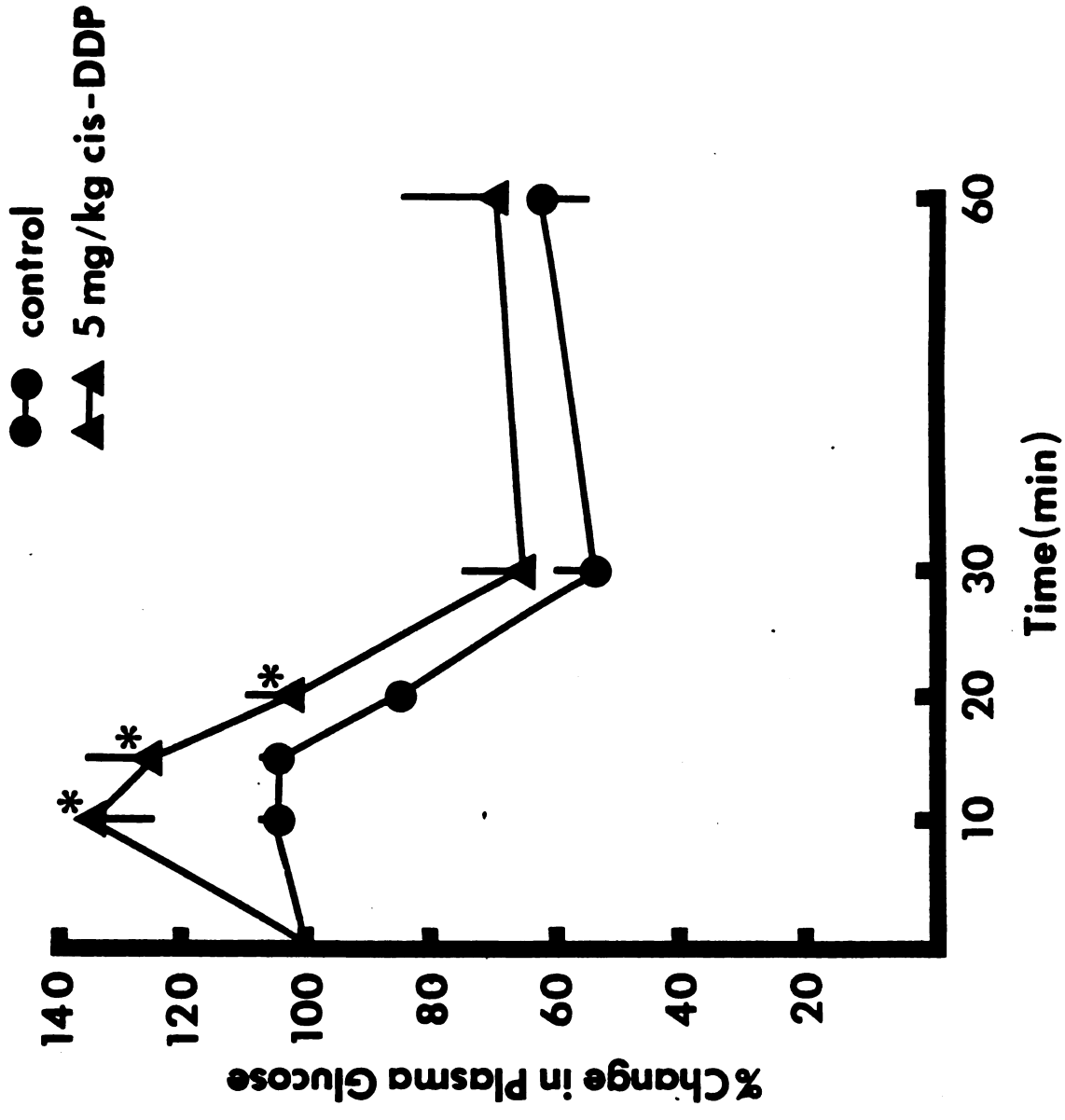

PLASMA GLUCOSE (g/dl-min)
30
25
20
15
10
5
*†
control
2.5
5
7.5
pair-fed
cis-DDP (mg/kg)

Figure 45

Figure 46. Effect of cis-DDP on glycemic response to exogenous insulin (0.28 IU/kg, ip). Experiments were conducted four days following cis-DDP (5 mg/kg) or vehicle administration. Controls were pair-fed to treated animals. Each point with vertical line represents the mean ± SEM of five determinations. Asterisks indicate a significant difference from controls at the corresponding time point ($p<0.05$).


control
5 mg/kg cis-DDP
% Change in Plasma Glucose
140
120
100
80
60
40
20
10
20
30
60
Time(min)
*
*
*

DISCUSSION

Glucose homeostasis is characterized by a balance between the opposing, but tightly coordinated, processes of glucose utilization and glucose production. Consequently, a chemical lesion or insult disturbing this delicate balance may result in hyperglycemia by impairing glucose utilization and/or increasing glucose production. Although regulation of these processes is primarily mediated by the "push-pull" actions of insulin and glucagon, the individual contributions of these hormones in producing hyperglycemia have been controversial. Recently, two schools of thought have emerged. The "unihormonal abnormality concept" proposes that hyperglycemia is the direct consequence of decreased glucose utilization associated with impaired insulin secretion and/or action. In contrast, the "bihormonal" or "double-trouble" hypothesis assigns to glucagon the role of an essential co-mediator (Unger and Orci, 1975). According to the latter postulate, hyperglycemia is not only a consequence of a relative or absolute deficiency of insulin, but in addition results from massive overproduction of glucose mediated by excessive circulating glucagon (Unger and Orci, 1975).

Although Unger's bihormonal theory remains controversial, it is generally agreed that impaired insulin metabolism plays an important role in glucose utilization and therefore may determine the severity of

hyperglycemia. Consequently, hyperglycemia may result, in part, from a lesion affecting any aspect of insulin metabolism, ranging from pancreatic beta cell damage to impaired insulin action at target tissues. With respect to the bihormonal theory, hyperglycemia may also result in part from alpha cell hypersecretion of glucagon and/or increased sensitivity to glucagon action at target tissues.

Several divalent metal cations influence carbohydrate metabolism by their ability to alter insulin and/or glucagon metabolism (Eaton, 1973; Ghafghazi and Mennear, 1975; Horak and Sunderman, 1975a,b; Ithakissios et al., 1975). Therefore, it was the central hypothesis of this dissertation that a divalent platinum compound, such as cis-DDP may similarly affect glucose homeostasis. Although the paucity of data documenting the effects of divalent metals on glucose homeostasis does not support a common single mechanism, hyperglycemia appears to be a common manifestation associated with metal toxicity (Ghafghazi and Mennear, 1973; Horak and Sunderman, 1975b).

Results of this study suggest that cis-DDP (5, 7.5 or 15 mg/kg) induces hyperglycemia in nonfasting animals (Figure 6). In contrast, administration of equimolar doses of trans-DDP did not result in hyperglycemia (Figure 6), indicating that this action is unique to the geometry of the cis-DDP complex. Furthermore, chemical properties other than the presence of a divalent platinum atom appear to contribute to cis-DDP hyperglycemia since administration of tetrachloroplatinate did not elevate blood glucose concentrations (Figure 6).

Treatment with 5 mg/kg cis-DDP resulted in hyperglycemia observed only in the nonfasting state and was no longer apparent upon fasting,

when exogenous (dietary) carbohydrate is removed. These results therefore suggest that decreased utilization of exogenous glucose contributes in part to cis-DDP hyperglycemia. To examine the effects of cis-DDP treatment on glucose utilization, glucose tolerance was evaluated by measuring plasma glucose following an exogenous glucose load. A glucose challenge in cis-DDP treated (5 mg/kg) animals resulted in a marked and persistent hyperglycemia (Figures 11 and 12), an effect consistent with impaired glucose utilization. Impaired glucose metabolism following treatment with 5 mg/kg cis-DDP was manifested to a modest degree on day 2 (Figure 11) and to a more profound extent on day 4 (Figure 12). However, this phenomenon appears to be transient as indicated by the negligible and complete absence of cis-DDP glucose intolerance on days 7 (Figure 13) and 14 (Figure 14), respectively. Although the transient nature of cis-DDP glucose intolerance is a pattern consistent with that of other divalent metals, its exact time course is markedly different. For example, glucose tolerance is impaired 1 hour following treatment with cadmium and returns to control values by 24 hours (Ghafghazi and Mennear, 1973). In contrast, at least four days are required for complete expression of cis-DDP glucose intolerance. This apparent delay of cis-DDP toxicity is not unique to the development of glucose intolerance; cis-DDP nephrotoxicity requires several days before histopathological or functional damage becomes apparent (Dobyan et al., 1980; Safirstein et al., 1981b; Ward and Fauvie, 1976). Thus, although the effects of cis-DDP on glucose tolerance are similar to those of other heavy metals, its delayed appearance may indicate that (1) the mechanisms mediating cis-DDP toxicity differ from those of other heavy metals

and/or (2) the biological handling of this platinum complex is uniquely different from metal salts. Both of these possibilities are consistent with the suggested biotransformation of cis-DDP to a more reactive and cytotoxic molecule (Long and Repta, 1981).

Neither trans-DDP nor tetrachloroplatinate impaired glucose tolerance at any time examined (Figures 11-16), indicating the specificity of action of the cis-DDP complex. Tissue platinum concentration per se probably does not account for this specificity since similar tissue distribution patterns of radiolabelled platinum have been reported following administration of cis-DDP or trans-DDP (Hoeschele and Van Camp, 1972). Although trans-DDP or tetrachloroplatinate may affect glucose metabolism prior to day 2, mechanisms mediating these acute changes are probably different than those mediating the more delayed actions of cis-DDP.

Since insulin plays a fundamental role in tissue uptake and metabolism of glucose, decreased utilization of exogenous glucose following cis-DDP treatment may be secondary to impaired insulin secretion and/or target tissue action. The deficient insulin response to a glucose stimulus 2 days following treatment with 7.5 mg/kg cis-DDP (Figure 9) is consistent with an impairment of insulin release by cis-DDP. However, plasma IRI concentrations following glucose stimulation in animals treated with 5 mg/kg cis-DDP was not significantly different from ad-libitum fed controls on days 2 (Figure 17), 4 (Figure 18), 7 (Figure 19) or 14 (Figure 20). Thus, although marked impairment of glucose utilization was manifested 2 days following treatment with either 5 mg/kg

(Figure 11) or 7.5 mg/kg (Figure 8) cis-DDP, plasma insulin response was only significantly impaired in the latter group.

This apparent discrepancy may be attributable to a dose-dependent impairment in insulin metabolism. Decreased glucose utilization in the 5 mg/kg cis-DDP group was evident despite an apparently normal plasma IRI response to a glucose stimulus, suggesting that cis-DDP may impair insulin action. Insulin resistance, by definition, exists when a known quantity of insulin produces a less than normal biological response (Olefsky, 1981); therefore, an apparently normal IRI response accompanied by decreased glucose utilization following cis-DDP treatment may suggest the presence of insulin resistance.

In uremia, impaired glucose utilization may be due to insulin resistance (DeFronzo, 1978; DeFronzo and Alvestrand, 1980; DeFronzo et al., 1981). Therefore, impaired glucose metabolism following cis-DDP treatment may be related to cis-DDP induced uremia. Impaired renal function in cis-DDP treated animals (5, 7.5 or 15 mg/kg) was characterized by polyuria (Figure 22), glycosuria (Figure 24) and elevated BUN concentrations (Figure 33). Since polyuria preceded polydipsia in treated (5 mg/kg) animals (Figure 22), increased water intake is probably secondary to volume depletion and is not a cause of increased 24 hour urine volume. Although polyuria following cis-DDP treatment may be a consequence of impaired concentrating ability (Safirstein et al., 1981b) or profound glycosuria (Figure 24), cis-DDP (5 mg/kg) glycosuria was transient (Figure 24) whereas polyuria persisted throughout the 14 day experiment, suggesting a dissociation between these two phenomena. Interestingly, those cis-DDP treated animals which were glycosuric were also hyperglycemic (Figure 6). Inasmuch as hyperglycemia

appeared to precede glycosuria following cis-DDP treatment, glycosuria may not only reflect a proximal tubular lesion, but may also be a consequence of hyperglycemia, conceivably by increasing the filtered load of glucose beyond the transport capacity for proximal tubular reabsorption. Additionally, the transient nature of cis-DDP glycosuria and hyperglycemia in surviving treated (5 mg/kg) animals further suggests an interrelationship between the two phenomena.

In contrast to cis-DDP, equimolar doses of the trans isomer did not affect urine volume (Figure 23) or urinary glucose excretion (Figure 25); indicating the specificity of cis-DDP action on the kidney. However, administration of 18 mg/kg tetrachloroplatinate resulted in both polyuria (Figure 23) and glycosuria (Figure 26) on days 1, 2, and 4. Similar to the effects of cis-DDP, polyuria preceded increased fluid intake in tetrachloroplatinate treated (18 mg/kg) animals, indicating that polyuria is not a consequence of increased fluid intake. Glycosuria accompanied polyuria at all times examined; therefore, the osmotic effect of increased urinary glucose may contribute to increased urine volume. Since tetrachloroplatinate did not result in hyperglycemia (Figure 6), glycosuria in treated (18 mg/kg) animals may primarily reflect impaired proximal tubular reabsorption glucose.

Since cis-DDP treated and pair-fed animals consumed equivalent quantities of food, urinary electrolyte excretion should be similar between the two groups unless cis-DDP selectively affects gastrointestinal absorption and/or renal excretion of electrolytes. Although cis-DDP (5 mg/kg) decreased urinary sodium (days 7 and 14 (Figure 29) and potassium concentration (days 2, 4, 7, and 14) (Figure 31) compared to pair-fed partners, the concomitant increase in 24 hour urinary volume in

treated animals resulted in renal electrolyte excretion equivalent to pair-fed animals. Only animals treated with 15 mg/kg cis-DDP exhibited increased urinary potassium excretion compared to pair-fed partners (Figure 31) and presumably resulted in net negative potassium balance. Similarly, neither trans-DDP nor tetrachloroplatinate affected urinary sodium and potassium excretion (Figures 30 and 32).

BUN concentrations were elevated following cis-DDP (5, 7.5 or 15 mg/kg) or tetrachloroplatinate (18 mg/kg), but not trans-DDP, treatment (Figure 33). cis-DDP induced elevation in BUN is a finding consistent with other reports (Leonard et al., 1971; Safirstein et al., 1981b; Schaeppi et al., 1973; Ward and Fauvie, 1976) and may be attributable to impaired renal clearance of urea, suggested by the decreased GFR in treated animals (Table 6). The decreased GFR may be related to the profound depression in effective renal plasma flow (C_{PAH}) (Table 6).

Although kidneys of animals treated with 5 mg/kg cis-DDP tended to exhibit a decreased capacity to accumulate PAH and TEA, no differences in these renal functions were noted compared to their pair-fed partners (Figure 34), suggesting that the observed effect was probably related to decreased food intake. In contrast, the presence of 500 or 600 µg/ml cis-DDP in incubation medium profoundly impaired the ability of renal cortical slices to accumulate both PAH and TEA (Figure 35). Insofar as PAH and TEA are secreted by independent systems, competitive inhibition for both transport carriers by cis-DDP is unlikely. Rather, cis-DDP related depression in organic ion accumulation is nonspecific and is probably related to a generalized depression in renal metabolism.

The apparent discrepancy between the profound depression in organic ion accumulation observed *in vitro* and the modest effects observed *in vivo* may be due, in part, to the relative differences in cis-DDP exposure in the two studies. Four days following cis-DDP treatment (5 mg/kg), kidneys of F-344 rats contain 10 µg platinum/g wet tissue (Litterst *et al.*, 1976b). Interpolation of these data suggest that kidneys of drug treated animals are exposed to 30-40 times less platinum than slices incubated in medium containing 500 or 600 µg/ml cis-DDP.

Taken collectively, these results indicate that administration of cis-DDP (5, 7.5 or 15 mg/kg), but not equimolar doses of trans-DDP, impairs specific renal functions. Therefore, the effects of cis-DDP on the kidney may contribute to the disturbances observed in glucose metabolism. Evidence supporting this postulation is based on the observation that administration of cis-DDP only at doses which are frankly nephrotoxic appear to impair glucose tolerance. Furthermore, BUN concentrations of the 5 mg/kg cis-DDP group were profoundly elevated on day 4 (Figure 33), a time at which glucose tolerance was also profoundly impaired (Figure 12).

Hemodialysis in uremic patients improves glucose tolerance and may be associated with improved tissue sensitivity to insulin (DeFronzo *et al.*, 1981). By analogy, if cis-DDP induced glucose intolerance were secondary to uremia, an amelioration in renal function should theoretically improve glucose metabolism. To determine the effect of cis-DDP induced uremia on glucose tolerance, the effects of mannitol pretreatment on cis-DDP nephrotoxicity and glucose intolerance were evaluated. Since mannitol pretreatment reduces cis-DDP nephrotoxicity without altering

its pharmacokinetic or therapeutic properties (Pera et al., 1979), any effect of this manipulation on cis-DDP glucose intolerance is presumably related to alterations in kidney function. Although mannitol pretreatment reduced plasma glucose concentrations in drug treated animals 30, 60 and 120 minutes following a glucose load, hyperglycemia relative to controls was still apparent at 15, 30 and 60 minutes (Figure 38). The partial improvement in glucose utilization by reducing cis-DDP nephrotoxicity suggests that impaired renal function contributes, at least in part, to cis-DDP glucose intolerance. Furthermore, mannitol pretreatment improved glucose tolerance with no apparent improvement in plasma IRI response to glucose (Figure 39), suggesting that improved glucose metabolism may be due to enhanced tissue sensitivity to IRI.

Although these results indicate that cis-DDP induced uremia may contribute to the observed glucose intolerance, administration of other agents which also induce uremia, i.e., cephaloridine, gentamicin or glycerol, did not uniformly impair glucose tolerance (Figure 40). These results therefore suggest that cis-DDP induced glucose intolerance is probably independent of uremia.

To determine if cis-DDP produces insulin resistance, the hypoglycemic actions of exogenous insulin were evaluated. Theoretically, if insulin action were impaired at the target tissue level by cis-DDP, then administration of exogenous insulin to treated animals should result in a blunted hypoglycemic response. However, treated animals exhibited a maximal hypoglycemic response 30 minutes following insulin administration, an effect which was similar to controls (Figure 46).

Insulin resistance may be due to decreased sensitivity to insulin (i.e., a shift in the dose-response curve to the right) and/or decreased maximal response to insulin (Kahn, 1978; 1980). Since only 10% of receptor occupancy is required for maximal response to insulin (Kahn, 1978), a reduction in receptor binding of up to 90% produces decreased sensitivity with no change in maximal response. Therefore, although maximal biological response to insulin was elicited with the given dosage of insulin, administration of smaller doses of insulin to treated animals may have produced a more blunted hypoglycemic response compared to controls. These results therefore do not rule out the possibility of decreased insulin sensitivity following cis-DDP treatment. Since mannitol pretreatment improved cis-DDP glucose intolerance without improving IRI secretion, the increased metabolism of glucose may be related to enhanced tissue sensitivity to insulin.

An alternative explanation for decreased glucose utilization accompanying an apparently normal plasma IRI response in cis-DDP treated animals (5 mg/kg) may relate in part to the presence of a less biologically active, although immunoreactive, beta cell secretory product. The insulin antibody used in the routine immunoassay cross-reacts with proinsulin (Rubenstein et al., 1968). Proinsulin in the normal state comprises only 5% of total plasma IRI concentration; therefore, cross-reactivity with this molecule does not usually confound interpretation of IRI data. However, in renal failure, fasting proinsulin concentrations are elevated 7.5-fold (Baba et al., 1979), a finding consistent with the primary role of the kidney in proinsulin degradation (Constan

et al., 1978; Izzo et al., 1978; Jaspan et al., 1977; Katz and Rubenstein, 1973; Kitabchi, 1977). The renal handling of proinsulin and insulin are characterized by high extraction rates (36 and 40%, respectively) and low urinary clearances, suggesting that almost all of the polypeptide extracted is metabolized by the kidney (Katz and Rubenstein, 1973). Despite these similarities, renal clearances of insulin and proinsulin represent one-third and two-thirds of their total metabolic clearance, respectively; the differential between the two polypeptides is related to the significant metabolism of insulin, but not proinsulin, by the liver (Katz and Rubenstein, 1973).

In renal failure, plasma proinsulin levels are also elevated following glucose stimulation and represent a significantly large percentage of total circulating IRI (Baba et al., 1979). On this basis, plasma IRI of cis-DDP treated animals in both the fasting and glucose-stimulated states may represent a disproportionately large amount of proinsulin, an effect secondary to diminished renal degradation associated with cis-DDP nephrotoxicity. If this were the case, then the less potent hypoglycemic actions of this beta cell product may explain: (1) fasting hyperglycemia accompanied by elevated plasma IRI in the 7.5 mg/kg cis-DDP group (Table 10) and (2) decreased utilization of exogenous glucose despite an apparently normal plasma IRI response in animals treated with 5 mg/kg cis-DDP (Figures 11, 12, 17 and 18). Since experiments were not designed to examine the composition of plasma IRI following cis-DDP treatment, these interpretations remain speculative.

In summary, these results indicate that cis-DDP (5 mg/kg) hyperglycemia accompanied by an apparently normal plasma IRI response to glucose,

may either reflect decreased tissue sensitivity to insulin and/or the presence of a less biologically active, although immunoreactive, beta cell secretory product. However, it might be argued that although absolute plasma IRI concentrations were apparently similar between treated and controls, they were deficient relative to the elevated blood glucose concentrations in animals treated with 5 mg/kg cis-DDP. Thus, the absence of an exaggerated IRI response to glucose following cis-DDP treatment suggests that impaired insulin synthesis and/or secretion contributes significantly to the observed impairment in glucose utilization (Figure 9). Although biosynthetic rates of insulin were not determined, pancreatic IRI concentrations were not affected by cis-DDP treatment (Figure 43), indicating that pancreatic stores of IRI are not deficient. Thus, impaired glucose utilization following exogenous glucose is probably related to impaired insulin secretion. Several factors may contribute to impaired insulin secretion following cis-DDP treatment. Starvation is known to result in glucose intolerance by impairing insulin release (Hedeskov, 1978) and therefore, reduced food intake following cis-DDP treatment (Figure 4) may contribute, in part, to impaired glucose metabolism. However, inasmuch as animals pair-fed to drug treated partners did not exhibit glucose intolerance (Figures 11-14), the decreased utilization of exogenous glucose following cis-DDP treatment appears to be independent of starvation. Furthermore, animals pair-fed to the 7.5 mg/kg group did not exhibit a blunted plasma IRI response to glucose stimulation (Figure 9), indicating that reduced food intake of drug treated animals did not significantly affect insulin

metabolism. Similarly, the relative response in plasma IRI from the fasting to the glucose stimulated state at 15 minutes was not significantly affected in animals pair-fed to the 5 mg/kg cis-DDP group (Table 3).

An impairment in insulin secretion may also result from drug-induced stress. The stress-induced release of adrenal catecholamines and glucocorticoids produces hyperglycemia; the former by a direct adrenergic effect on pancreatic islet function, resulting in inhibition of insulin release, and the latter by decreasing receptor binding to insulin. Consequently, impaired glucose utilization may not necessarily result from a direct insult to pancreatic islet function; but rather to changes in insulin metabolism effected by a stress related increase in catecholamines and glucocorticoids. In addition to influencing pancreatic islet functions, catecholamines may also directly activate hepatic glycogenolysis and gluconeogenesis or inhibit tissue uptake of glucose.

These well documented effects of adrenal catecholamines and glucocorticoids on carbohydrate metabolism coupled with the observation of adrenal medullary and cortical hypertrophy following cis-DDP treatment (Toth-Allen, 1970), suggest that cis-DDP glucose intolerance may be adrenal mediated. The adrenal weight to body weight (AW/BW) ratios were elevated 4 days following treatment with 5 mg/kg cis-DDP (Table 4), an effect which is not due to decreased body weight since these ratios were significantly different from pair-fed partners of comparable body weight (Figure 5). Furthermore, changes in relative adrenal weights (Table 4) were accompanied by changes in glucose tolerance (Figures 12 and 14) 4 and 14 days following treatment with 5 mg/kg cis-DDP.

Theoretically, if glucose intolerance following cis-DDP treatment were adrenal mediated, then adrenalectomy should prevent these abnormalities in carbohydrate metabolism by improving insulin metabolism. Bilateral adrenalectomy reduced plasma glucose concentrations in both the fasting state and 120 minutes following a glucose load in cis-DDP treated animals (Figure 21). This improvement in glucose metabolism by adrenalectomy appears to be related to enhanced tissue sensitivity to insulin as indicated by the absence of fasting hyperglycemia (Figure 21) and hyperinsulinemia (Table 5) in the Adx/cis-DDP group. Enhanced tissue sensitivity to insulin in adrenalectomized animals may be due to increased receptor binding affinity to insulin associated with the presumed absence of glucocorticoid activity (Kahn, 1978). Nevertheless, impaired glucose utilization following exogenous glucose administration was apparent during the early stages of the glucose tolerance test (15, 30, and 60 minutes) in both cis-DDP groups, regardless of the presence or absence of adrenal glands (Figure 21). These results sugest that although partial improvement in cis-DDP glucose intolerance was observed, a stress related effect on catecholamine and/or glucocorticoid metabolism cannot entirely account for the abnormalities in carbohydrate metabolism. Therefore, factors in addition to those related to adrenal function must mediate cis-DDP glucose intolerance.

One of the critical events in glucose stimulated insulin release is an increased flux of ionized calcium into the cytosol of pancreatic beta cells. Diminished insulin response to glucose has been reported in hypocalcemia (Bansal et al., 1975; Gero et al., 1976), an effect which

may relate to the dependence of insulin secretion upon extracellular calcium. Hypocalcemia may result from cis-DDP treatment (Hayes et al., 1979) and therefore may contribute to glucose intolerance. However, serum calcium concentrations of drug treated animals were comparable to controls (Table 9). Hypokalemia is also known to result in impaired insulin secretion (Rowe et al., 1980); however, serum potassium concentrations were also unaltered by cis-DDP treatment (Table 9). Although neither serum potassium nor calcium concentrations were affected by cis-DDP treatment, an effect on their beta cell metabolism cannot be discounted.

Alternatively, impaired insulin secretion following cis-DDP treatment may be a result of a direct pancreotoxic effect of the drug. However, administration of cis-DDP at doses known to impair glucose metabolism did not result in histopathological damage to the pancreas, as indicated by the absence of islet degeneration, necrosis and inflammation using light microscopy (Figure 41). Although no gross histopathological damage to the pancreas was evident, cis-DDP may alter islet cell population and/or subcellular architecture. Further studies would be needed to explore these aspects. Serum amylase activity, which provides a gross estimate of pancreatic function, was not affected by cis-DDP treatment (Figure 42), indicating the unlikelihood of cis-DDP induced pancreatic damage.

Since glucagon has been assigned the role of an essential co-mediator in diabetes (Unger and Orci, 1975), it was hypothesized that hyperglycemia following cis-DDP treatment may also be mediated in part by drug induced hyperglucagonemia. Glucagon may induce hyperglycemia

by increasing endogenous glucose production beyond the capacity for glucose utilization. Plasma IRG concentrations were elevated following cis-DDP treatment (5 or 7.5 mg/kg) and accompanied the observed glucose intolerance (Figures 10, 17 and 18). Furthermore, administration of 7.5 or 10 mg/kg cis-DDP induced hyperglycemia in both the fasting and nonfasting state (Figures 6 and 7), indicating the likelihood of endogenous glucose overproduction.

Endogenous glucose overproduction may be characterized by increased rates of glycogenolysis and/or gluconeogenesis. Although fasting hyperglycemia accompanied by hyperglucagonemia, was evident following treatment with 7.5 mg/kg cis-DDP, increased hepatic and renal activities of the gluconeogenic enzymes, glucose-6-phosphatase and fructose-1,6-diphosphatase, were not observed (Table 10). Several possible explanations may account for the absence of increased gluconeogenesis accompanying fasting hyperglycemia following cis-DDP treatment (7.5 mg/kg). Mechanisms mediating fasting hyperglycemia may not involve an absolute increase in glucose production; rather, glucose production may be increased only relative to rates of glucose utilization. Since cis-DDP treatment impairs glucose utilization, normal rates of glucose production may exceed the capacity for utilization. Further studies would be needed to determine the relative rates of glucose production and utilization following cis-DDP. Secondly, glucagon mediated glucose production is a short-lived phenomenon, resulting in only a transient increase in glucose production (Fradkin et al., 1980). Therefore, since glucose utilization is impaired by cis-DDP, a brief burst in glucose production (prior to day 4) may result in prolonged hyperglycemia unaccompanied by

a sustained elevation in glucose production. Finally, an alternative explanation may be that fasting hyperglycemia in treated animals is mediated by an increased glucose production due either to a preferential mobilization of glycogen stores or to increased gluconeogenesis which was for some reason not detected in the present experiments.

In contrast to the 7.5 mg/kg cis-DDP group, hyperglucagonemia following treatment with 5 mg/kg cis-DDP was not associated with fasting hyperglycemia (Table 10). Although it may be argued that profound glycosuria induced by cis-DDP (Figure 24) may mask the hyperglycemic actions of glucagon, hyperglycemia was evident in these animals in the nonfasting state (Figure 6). Thus, the paradoxical appearance of hyperglucagoemmia and normoglycemia is probably not related to urinary losses of glucose. Rather, this phenomenon may be attributed to the effects of cis-DDP on the biological activities of circulating IRG and/or to metabolic adaptation to glucagon action. Plasma glucagon immunoreactivity comprises at least four different components with differing molecular weights, averaging 150,000, 9000-12,000, 3500 and 2000 daltons (Jaspan et al., 1981). The 3500 MW form corresponds with "true pancreatic" glucagon and is believed to be the biologically active form of glucagon. In contrast, the nature of the higher MW forms of plasma IRG are not well defined, although the 9000-12,000 MW fraction may represent a precursor (proglucagon) or intermediate of glucagon biosynthesis. The marked heterogeneity of plasma IRG is further complicated by the presence of glucagon like immunoreactive (GLI) components, which are principally of intestinal origin.

Since the antibody used to measure plasma IRG in these studies cross-reacts with all immunoreactive components of glucagon, it was hypothesized that the elevation in plasma IRG following cis-DDP treatment (5 or 7.5 mg/kg) may represent a form other than the 3500 MW. To test this, plasma IRG was quantified using an antibody which cross-reacts with all IRG components ("total") and one which cross-reacts with only the 3500 MW form ("true pancreatic"). Although plasma concentrations of "true pancreatic" IRG of the 5 mg/kg group were comparable to ad-libitum fed controls, they were significantly greater than pair-fed controls (Table 11). "Extra-pancreatic" plasma IRG ("total" - "true pancreatic") of animals treated with 5 or 7.5 mg/kg cis-DDP represented a significantly large proportion of total circulating IRG (70 and 84%, respectively) compared to ad-libitum or pair-fed controls (43 and 38%, respectively). These data suggest that elevated plasma IRG following cis-DDP treatment is primarily due to an increase in a form other than the 3500 MW component. Normoglycemia accompanied by an elevation in "extra pancreatic" plasma IRG in animals treated with 5 mg/kg cis-DDP might suggest decreased hyperglycemic activity of this IRG component. However, this suggestion remains speculative since the biological activity of these IRG fractions was not determined in these studies.

Despite the relatively large proportion of "extra pancreatic" plasma IRG in the 7.5 mg/kg group, "true pancreatic" plasma IRG concentration was elevated 5 to 7 fold (Table 11). The magnitude of this elevation would be expected to produce severe fasting hyperglycemia and massive increases in glucose production. However, neither of these phenomena

was observed, suggesting the possibility of metabolic adaptation to the biological actions of "true pancreatic" glucagon following cis-DDP treatment. The effects of hyperglucagonemia are short-lived; thus, a chronic and sustained elevation in circulating glucagon is not characterized by continued glucose overproduction (Fradkin et al., 1980; Sherwin and Felig, 1980). Adaptation to chronic hyperglucagonemia is well documented and appears to be characterized by either down regulation of glucagon receptors (Bhathena et al., 1978) and/or the development of hepatic refractoriness to the enzymatic effects of glucagon (e.g., decreased activity of adenylate cyclase, increased activity of phosphodiesterase) (Fradkin et al., 1980). Regardless of the exact mechanism, it is likely that chronic hyperglucagonemia following cis-DDP treatment results in metabolic adaptation and this may account for the absence of severe fasting hyperglycemia and glucose overproduction normally elicited by an elevation of glucagon.

An alternative, but less likely, explanation for hyperglucagonemia unaccompanied by severe fasting hyperglycemia following cis-DDP treatment, is the possibility of an impaired glycemic response to glucagon at the target tissue level. This phenomenon has been reported to contribute to the hyperglucagonemia and normoglycemia of cobalt chloride treated animals (Eaton, 1973). To test this possibility in cis-DDP treatment, animals were evaluated for their glycemic response to exogenous glucagon. A blunted response to glucagon was not observed in treated animals (Table 13); therefore, glucagon resistance at the target tissue level is unlikely following cis-DDP treatment. In fact, animals treated with 7.5 mg/kg cis-DDP demonstrated marked hyperglycemia 20 and 30 minutes following glucagon administration (Table 13). Since the

maximal increase in blood glucose was not different between treated and control animals, the hyperglycemia following treatment with 7.5 mg/kg cis-DDP probably reflects decreased tissue uptake and metabolism of glucose rather than continued increase in glucose production.

In summary, these results indicate that the absence of severe fasting hyperglycemia and massive glucose overproduction in cis-DDP hyperglucagonemia may be related in part to the biological activities of the plasma IRG components and/or to the development of metabolic adaptation to a chronic and sustained elevation in plasma IRG.

It is likely that cis-DDP hyperglucagonemia is due to decreased degradation of IRG rather than increased secretion. The kidney represents the principal site of glucagon degradation as indicated by the relatively high extraction and low urinary clearance of this hormone (Bastl et al., 1977; Lefebvre et al., 1974). Furthermore, hyperglucagonemia, independent of pancreatic IRG secretion, has been documented following acute bilateral kidney exclusion (Lefebvre and Luyckx, 1976), renal artery clamping (Lefebvre et al., 1974), bilateral ureteral ligation (Bilbrey et al., 1974; Emmanouel et al., 1976), 70% nephrectomies (Bastl et al., 1977) and in chronic renal failure patients in which hyperglucagonemia is reversed by renal transplantation (Bilbrey et al., 1975). In this manner, hyperglucagonemia of renal failure has been attributed to impaired renal degradation rather than increased alpha cell secretion. A similar mechanism associated with cis-DDP nephrotoxicity may therefore mediate cis-DDP hyperglucagonemia.

Reduced cis-DDP nephrotoxicity by mannitol pretreatment also resulted in reduced plasma IRG concentrations in treated animals (Figure 39 and Table 7). Furthermore, administration of other agents which impair renal function, such as glycerol and gentamicin, also resulted in an elevation in plasma IRG (Table 12). These results are consistent with the postulate that cis-DDP hyperglucagonemia is primarily mediated by impaired renal degradation of glucagon, an effect secondary to cis-DDP nephrotoxicity.

The mechanisms by which glucagon is handled by the kidney appear to involve glomerular filtration followed by proximal tubular reabsorption (Nahara et al., 1958); the proximal tubules contain glucagon degrading enzymes located in both the brush border and cytosol (Duckworth, 1976a). Kuku et al. (1975) reported that hyperglucagonemia of chronic renal failure was largely due to a striking elevation in a 9000 MW component, comprising 57% of total circulating IRG, and a moderate elevation in the 3500 MW fraction. The 3500 MW form appears to be handled primarily by glomerular filtration and tubular reabsorption whereas the renal handling of the 9000 MW component may involve peritubular uptake from postglomerular capillaries (Emmanouel et al., 1976).

These results, when extrapolated to the present studies, suggest that the moderate elevation in the 3500 MW form of IRG may be related to reduced GFR of drug treated animals (Table 6). Since the 3500 MW IRG component is the only IRG peptide to be metabolized by both kidney and liver (Jaspan et al., 1977), hepatic metabolism of this component in cis-DDP treated animals may prevent a profound elevation in the circulation. In contrast, the kidney is the major site of catabolism

of the 9000 MW form of IRG. Therefore, the presumed impairment in renal degradation of IRG associated with cis-DDP nephrotoxicity may result in increasing circulating levels of this IRG component in treated animals. Although experiments were not designed to identify the immunoreactive components of plasma IRG in cis-DDP treatment, it seems reasonable to speculate that a large proportion of "extra pancreatic" plasma IRG in treated animals is represented by the 9000 MW form.

An alternative explanation for cis-DDP hyperglucagonemia is increased glucagon secretion. Glucagon hypersecretion from the alpha cells of the pancreas may be secondary to catecholamine release associated with drug induced stress. If such a mechanism mediates cis-DDP hyperglucagonemia, then adrenalectomy should prevent cis-DDP hyperglucagonemia. However, since plasma IRG concentrations were not significantly elevated 2 days following cis-DDP treatment (Table 5), it is difficult to evaluate the role of adrenal mediated stress in cis-DDP hyperglucagonemia.

In summary, these results indicate that cis-DDP treatment results in profound hyperglucagonemia, an effect which is probably related to decreased renal degradation of IRG. Since hyperglucagonemia in treated animals was not associated with severe fasting hyperglycemia, the biological activity of this hormone and its contribution to cis-DDP glucose intolerance must be questioned. Several lines of evidence suggest that cis-DDP hyperglucagonemia probably does not mediate cis-DDP glucose intolerance: (1) hyperglucagonemia was apparent on days 2, 4, and 7 (Figures 17-19) following treatment with 5 mg/kg cis-DDP, yet glucose tolerance was impaired only on days 2 and 4 (Figures 11-14), (2) although

treatment with cis-DDP, gentamicin and glycerol resulted uniformly in hyperglucagonemia (Table 12), glucose tolerance was differentially affected by these agents (Figure 40), and (3) hyperglucagonemia was not associated with fasting hyperglycemia in animals treated with 5 mg/kg cis-DDP. These results therefore suggest that hyperglucagonemia per se does not necessarily result in impaired glucose tolerance. Although glucagon may contribute to cis-DDP hyperglycemia by acutely increasing glucose production, the abnormalities in carbohydrate metabolism persist for at least several days following cis-DDP treatment, indicating that other mechanisms must be invoked. It is likely that the primary lesion mediating cis-DDP hyperglycemia involves impaired glucose utilization associated with impaired insulin secretion. The exact mechanisms underlying the impairment in insulin secretion by cis-DDP treatment cannot be evaluated from these studies and therefore merit further investigation.

SUMMARY

This study was designed to characterize the effects of cis-DDP and other divalent platinum compounds on carbohydrate metabolism and to elucidate the biochemical and endocrine correlates mediating these metabolic abnormalities.

Glucose metabolism was evaluated in male F-344 rats treated with equimolar platinum doses of cis-DDP, trans-DDP or ammonium tetrachloroplatinate. Since preliminary studies indicated a dose-related anorexia associated with cis-DDP treatment, a pair-fed control group was also studied to correct for the metabolic effects of reduced food intake. Administration of cis-DDP, but neither trans-DDP nor tetrachloroplatinate, resulted in fasting (7.5 or 10 mg/kg) and nonfasting (5, 7.5 or 10 mg/kg) hyperglycemia. Impaired glucose utilization appears to contribute to cis-DDP hyperglycemia as indicated by the marked and persistent hyperglycemia following exogenous glucose administration in treated (5 or 7.5 mg/kg) animals. Impaired glucose utilization was apparent 2 and 4, but not 7 and 14, days following treatment with 5 mg/kg cis-DDP, indicating that it is a transient phenomenon. Neither trans-DDP nor tetrachloroplatinate impaired glucose tolerance at any time examined. These results suggest that glucose intolerance following cis-DDP treatment is unique to the geometry of the complex and that properties other than the presence of a divalent platinum atom must contribute to cis-DDP glucose intolerance.

The mechanisms mediating cis-DDP glucose intolerance are related to impaired insulin metabolism. Decreased utilization of exogenous glucose was observed in treated (5 mg/kg cis-DDP) animals despite an apparently normal plasma IRI response. Although amelioration of cis-DDP induced uremia was associated with an improvement in cis-DDP glucose intolerance, other nephrotoxicants which induced uremia did not uniformly impair glucose tolerance. These results therefore suggest that cis-DDP glucose intolerance is mediated by mechanisms other than those associated with uremia. Although improvement in glucose metabolism by reducing cis-DDP nephrotoxicity suggests that cis-DDP impairs insulin action at target tissues, treated animals did not exhibit a blunted hypoglycemic response to exogenous insulin. Although plasma IRI concentrations following glucose stimulation were similar between control and treated animals, it is likely that the composition of plasma IRI is different following cis-DDP treatment. cis-DDP impairs renal function; therefore, renal degradation of proinsulin, which is a less biologically active, although immunoreactive, beta cell secretory product, may be compromised in treated animals and therefore may constitute a large proportion of plasma IRI. Further studies would be needed to explore this possibility.

Although it is likely that proinsulin is elevated following cis-DDP treatment, an exaggerated total plasma IRI response to glucose stimulation was not apparent, indicating that cis-DDP glucose intolerance is primarily mediated by impaired insulin release. Pancreatic concentrations of IRI were not significantly affected by cis-DDP treatment; therefore, pancreatic insulin depletion probably does not account for

impaired release. Impaired insulin release appeared to be independent of starvation, uremia and adrenal-mediated stress. Furthermore, neither hypocalcemia nor hypokalemia was evident in treated animals; therefore, glucose intolerance associated with a blunted insulin response cannot be attributed to these phenomena. A direct pancreotoxic effect of cis-DDP also seems unlikely as indicated by the normal appearance of the pancreas upon histological examination by light microscopy following cis-DDP treatment.

Since glucagon is known to contribute to hyperglycemia by increasing glucose production, glucagon metabolism was evaluated following cis-DDP treatment. cis-DDP glucose intolerance was accompanied by hyperglucagonemia. However, several lines of evidence indicate that the elevation in plasma IRG may not directly mediate cis-DDP glucose intolerance: (1) hyperglucagonemia following cis-DDP treatment did not increase hepatic or renal G6Pase or FDPase activities, suggesting an absence of glucagon mediated glucose production, (2) a form other than the 3500 MW form of plasma IRG constituted a large proportion of total plasma IRG in treated animals, (3) animals treated with 5 or 7.5 mg/kg cis-DDP exhibited a significant increase in the 3500 MW form of IRG; however, this was not accompanied by severe fasting hyperglycemia and (4) the biological effects of glucagon are known to be short-lived; therefore, chronic hyperglucagonemia of treated animals may not result in continued increase in glucose production.

The elevation in plasma IRG following cis-DDP treatment is probably related to decreased renal degradation of this hormone. This is suggested

by the decrease in plasma IRG when cis-DDP nephrotoxicity is reduced by mannitol pretreatment. Furthermore, other nephrotoxicants such as gentamicin and glycerol also resulted in hyperglucagonemia.

In conclusion, cis-DDP treatment results in hyperglycemia, an effect mediated by impaired utilization of glucose associated with a blunted insulin release. The exact mechanisms mediating the deficient insulin release cannot be evaluated from these studies and merit further investigation.

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