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Metabolic Fate and Evaluation of Injury in Rats and Dogs Following Exposure to the Hydrolysis Products of Uranium Hexafluoride

Implications for a Bioassay Program Related to Potential Releases of Uranium Hexafluoride
Report Period: July 1979 - October 1981

Prepared by P. E. Morrow, L. J. Leach, F. A. Smith, R. M. Gelein, J. B. Scott, H. D. Beiter, F. J. Amato, J. J. Picano, C. L. Yuile, T. G. Consler

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Prepared for
U.S. Nuclear Regulatory Commission

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ABSTRACT

This final report summarizes the experimental studies undertaken in rats and dogs in order to help provide adequate biological bases for quantifying and evaluating uranium hexafluoride (UF_6) exposures. Animals were administered the hydrolysis products of UF_6 by inhalation exposures, intratracheal instillations and intravenous injections. Attention was given to dose-effect relationships appropriate to the kidney, the unique site of subacute toxicity; to the rates of uranium excretion; and to uranium retention in renal tissue. These criteria were examined in both naive and multiply-exposed animals. The findings of these studies partly substantiate the ICRP excretion model for hexavalent uranium; generally provide a lower renal injury threshold concentration than implicit in the MPC for natural uranium; indicate distinctions in response (for example, uranium excretion) are based on exposure history; compare and evaluate various biochemical indices of renal injury; raise uncertainties about prevailing views of "reversible" renal injury, renal "tolerance" and possible hydrogen fluoride synergism with uranium effects; and reveal species differences in several areas, for example, renal retention of uranium. While these studies present some complicating features to extant bioassay practice, they nevertheless supply data supportive of the bioassay concept.

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PREFACE

This final report describes the animal studies undertaken from 1978 to 1981 at the University of Rochester on the hydrolysis products of UF_6, namely UO_2F_2 aerosol and HF gas. An interim report, NUREG/CR-1045, (Morrow et al., 1980) entitled "Acute Effects of Inhalation Exposure to Uranium Hexafluoride and Patterns of Deposition, UF_6/UO_2F_2 Studies in Experimental Animals, June 1978 - July 1979" was published in August 1980 after the first year of the study. Some peer-reviewed publications on several aspects of these studies are in various stages of preparation. However, this final report summarizes the experimental results found in both rats and dogs for the entire study period and provides a summary and conclusions of special relevance to the NRC bioassay program for UF_6.

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I. SUMMARY AND CONCLUSIONS

The summary and conclusions described in the interim report (NUREG/CR-1045, Morrow et al., August 1980) and those derived from the more recent studies are in general agreement. Most of the original statements were extended or amplified by the newer data, and in a few instances, new summarizing statements and their interpretations were prepared. Collectively, these are presented in the following section together with special remarks regarding NRC bioassay and action level concepts appropriate to UF_6 exposures.

(1) Renal Excretion of Uranium: The principal route of excretion of absorbed UO_2F_2 is renal. Uranium excretion by UO_2F_2-exposed rats differed from dogs in that the total urinary U elimination rarely exceeded 60 percent of the absorbed dose (mean 53 percent) and the daily excretion rate did not follow the ICRP power function as well. The urinary U excretion rate in the dog can be correlated closely with the total urinary uranium excretion and this follows nearly exactly the current ICRP excretion model viz., 0.80 urinary uranium excretion in the first 24 hours, compared to 0.82 in the dog, and the remaining fraction, 0.20, excreted as a power function $t^{-1.5}$ where t is expressed in days, compared to $0.18t^{-1.5}$ in the dog. Also in the dog, these expressions can be changed to fractions of the total absorbed dose by reducing 0.82 to 0.63 and decreasing $0.18t^{-1.5}$ to $0.14t^{-1.5}$, inasmuch as the total urinary elimination of uranium bears a predictable relation to total absorbed dose in naive (not previously exposed) dogs, i.e. 0.78.

(2) Fecal Elimination of Uranium: Neither the retention nor the excretion of uranium can be related easily to total exposure or total uranium deposition since these are particle size and species dependent. The present study indicates this problem quite well when nose-exposed rats are compared with dogs exposed through endotracheal tubes and when rats are exposed to UO_2F_2 aerosols of different size. A similar problem exists when comparing nose-exposed animals to intratracheally-instilled or intravenously-injected animals. The problem is partly specie-dependent (see 1) but is mainly due to the initial deposition pattern, which in the nose-exposed animal, always infers significant nasal airway deposition. This nasal deposit, plus a lesser amount translocated from the tracheobronchial airways (and any uranium ingested from grooming) is transported to the gastrointestinal tract well before significant absorption occurs, and in the gastrointestinal tract, uranyl fluoride experiences very limited absorption. Nearly two-thirds of the inhaled UO_2F_2 was, on the average, translocated to the rat gut and nearly all of this appeared in the feces (~64 percent of inhaled UO_2F_2). For the exposure conditions used, this was quite consistently found in these rats.

Evidence is also presented that a small amount of the absorbed (or injected) UO_2F_2 dose appears in the canine bowel, so detectable fecal uranium levels (3-5 percent of the absorbed dose) probably arise from biliary secretion of uranium. This contribution to fecal

U is expected to be obscured, in the case of inhalation exposure, by the greater amount associated with upper airway deposition (and ingestion). In the intratracheally-dosed rats, by comparison, 1.5 percent of their instilled uranium was recovered in the feces (0-4 days). It is not clear if this was translocated material from the bronchial airways or possibly biliary secretion, but the similarity to the intravenous dog data is obvious.

The new ICRP model for particle deposition (ICRP 1979) should not overestimate the probable nasal deposition of UO_2F_2 for a given particle size, but may underestimate it due to the hygroscopic character of UO_2F_2. The fecal uranium output derived therefrom, might be useful as an index of UO_2F_2 exposure, but it will offer little predictive information about the effective (systemic) dose or urinary levels unless appropriate particle size data are obtained conjointly.

(3) Uranium Retention in the Lungs: If the exposure of UO_2F_2 is limited to the lungs, as it was with the intubated dogs and the rats receiving intratracheal instillations, then the intake is tantamount to uptake since lung absorption is very rapid and nearly complete. These efficient, quantitative exposure techniques totally by-passing the nasal airways were designed to optimize dosing. In any case, pulmonary retention of UO_2F_2 in the dog was mainly (~90 percent) described by a 0.3 hr half-time. This is much more rapid than for uranium trioxide (dominant half-time 4.7 days) another U^{+6} compound previously studied in the dog; hence, uranyl fluoride may not be prototypic of other U^{+6} compounds in the lungs.

In rats exposed to UO_2F_2 by inhalation, it was impractical to detail the early lung retention by external counting. At necropsies, there was, nevertheless, evidence for a small persistent fraction (0.05) of the initial lung burden having a half-time of days. This was also seen in the rodent intratracheal studies. A similar persistence of uranium was not noted in other soft tissues of the rat exclusive of the kidneys. These findings compare with a 0.04 fraction of the initial lung burden in the dog associated with a 2 day retention half-time, based on 0-6 day external counting data. The dog kidneys were the only other measurable soft tissue retention site for uranium.

Intratracheally-dosed rats support the inhalation data in both species with the finding that pulmonary absorption of UO_2F_2 is prompt and nearly complete. The less than 0.02 fraction retained by the lungs from the intratracheal study rats was measurable for several weeks. Thus a small but relatively long-term retention component for uranium in the lungs is established, although its half-time is uncertain. Other investigations on this point indicate small but prolonged soft tissue retention of uranium (op cit). These findings may have important implications in chronic exposure circumstances.

(4) Uranium Retention in Kidneys and Bone: Renal retention of uranium following either UO_2F_2 injection or inhalation in the dog was basically the same i.e., peaking before 6 hours and then

declining with an apparent 9.3 day half-time. The limitation of a 19-day study following UO_2F_2 administration doubtlessly causes the canine renal retention to be underestimated. The new dog data of Stevens et al. with intravenous uranyl citrate and the new UO_2F_2 data from our dog studies are essentially identical from 1-19 days, but the long-term data from Stevens et al. reveal a correspondingly longer biological half-time of 79.5 days for ~2/3 of the initial renal burden. After single administration of soluble uranium in the present dog study and in the dog study of Stevens et al., peak uranium levels in the kidneys suggest an f_2' value of ~ 0.25*. The Stevens et al., data indicate 0.15 as the renal fraction associated with the 79.5 day half-time.

The rat studies of renal retention were of longer duration, consequently, the 17.3 day half-time determined for uranium retention in the kidneys of rats followed up to 38 days after UO_2F_2 inhalation was the longest retention time measured in naive animals. In the rat study of intratracheally-administered UO_2F_2, a similar renal retention half-time of 16.7 days was obtained over a 60 day period.

The retention half-times for uranium in the rat kidneys support generally the 15 day value used in the MPC (sol U) computation. However, evidence for a more complex and protracted renal retention of U^{+6} was found in multiply-exposed rats (See 5) and has been reported by others.

On the basis of the first week's pattern of uranium distribution following UO_2F_2 administration, the rat kidney and bone contained 74 and 20 percent of the body burden, respectively, whereas in the dog it was 71 and 26 percent, respectively, a very similar finding. These results indicate an f_2 value of ~0.25*. The limitations of our study for renal retention data also apply to the measured retention of uranium in the skeleton, only more so, because of its protracted nature. At early postexposure times, the bone exchanges cations, including UO_2^{++}, consequently during the several-day, dynamic phase, the longer term retention of uranium is obscured. Notwithstanding, a skeletal retention function was measured in rats indicating a half-time of 63 days. Because of the limitations cited, the recently estimated 917 day half-time is believed to be the best experimentally determined retention value available for uranium.

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*f_2' is defined as fraction of radionuclide passing from blood to critical body organ; whereas f_2 is defined as the fraction of radionuclide in the critical organ of that in the body. From ICRP Publication 2, 1960.

(5) Singly Versus Multiply-exposed Animals: Urinary uranium excretion in naive dogs was cited (See 1) as supporting the present ICRP excretion equations. Although the rat and dog differed quantitatively in this particular regard, both species were found to be consistent in the findings of increased renal uranium concentration and decreased urinary uranium excretion after multiple UO_2F_2 exposures. This was found in all of the rat and dog studies following inhalation or intravenous administration. Intratracheal studies in the rat also demonstrated an increased renal retention half-time for uranium of 32 days, or nearly twice that found in naive rats. Also the fact that uranium excretion appeared to be reduced by single high dose levels was reported in the Rochester and Boston studies in humans and this, too, was also seen in the rat studies. Paradoxically, this finding (decreased U excretion) was not seen after intratracheal administration of UO_2F_2 in rats, but increased renal retention of U was confirmed. Collectively, these findings indicate that worker exposure history is probably very important in determining urinary U excretion rates and renal U retention times.

(6) Renal Injury Threshold: Intravenous doses of 0.01 mg U kg^{-1} as UO_2F_2 were nephrotoxic to the dog when judged by urinary biochemical changes, viz., glucosuria. Inhalation and intravenous doses of ~0.1 mg U kg^{-1} produced many abnormal urinary biochemical changes in the dog, e.g., glucosuria, proteinuria, GFR depression, etc., and provided histopathologic findings of generalized renal tubular damage. In the rat, an intravenous dose of 0.1 mg U kg^{-1} produced transient elevations in several urinary biochemical indices of renal injury. These results and the earlier reports of findings of doses 0.07 mg U kg^{-1} (i.v. uranyl nitrate) being nephrotoxic in man, suggest species distinctions in susceptibility to uranium-induced renal injury with the dog being most susceptible, the rat least, and man intermediate. The specie distinctions are essentially quantitative, not qualitative.

On the basis of available human data and the renal levels associated with these minimally-injurious UO_2F_2 doses in dogs and rats, the 3 μg U g^{-1} kidney level is much greater (factor 5 to 10) than the peak renal concentration achieved in these multispecies experimental studies. Moreover, the 3 μg U g^{-1} renal concentration represents a sustained renal level (prolonged retention phase) as would be required by MPC calculations. The transient peak levels, therefore, belie the discrepancy between the previously-accepted and experimentally-determined threshold concentrations for renal injury in naive subjects from this study. The conclusion has implications to the 30 μg U l^{-1} urine action level. See Summary and Conclusions (10), following.

(7) Reversibility of Renal Injury: The "reversible" or "transient" nature of the urinary biochemical changes does not parallel the injury or repair processes in histopathologic specimens. Moreover, while many biochemical criteria return to "normal" during the first 14 days post-uranium administration, some do not, e.g., GFR. and other evidence of renal dysfunction may persist, e.g., loss of or

diminished urine concentrating function, for many weeks. This is a dose-related phenomenon. Even when the repair process is described histologically as well advanced, areas of recognizable injury persist, and the regenerated tubular epithelium appears basophilic and flattened. Consequently, we believe the past and present evaluations of renal function and renal toxicity of uranyl uranium, e.g. UO_2F_2, are reasonably consistent, but both have failed to quantify and characterize the pathophysiology of the injury and repair phases adequately. We conclude that descriptions of "reversible and transient" effects of uranium are euphemistic.

(8) Indices of Renal Injury: Indications of renal injury, viz., urinary protein, urinary glucose, urinary N-acetyl-glucosaminidase, urinary α-NH_2 nitrogen, urinary phosphate, urinary citrate, creatinine and inulin clearance (glomerular filtration rate), and plasma urea nitrogen, were found to have different response patterns, both quantitatively and temporally. Furthermore, the apparent sensitivity and persistence of these indicators differed in naive and previously-exposed animals. In general, responses to uranyl fluoride were qualitatively similar in rats and dogs, the major distinction being the fact that glucosuria was induced at lower injected doses in the dog, viz., 0.01 mg U kg^{-1} versus 0.1 mg U kg^{-1} in the rat. In both species an equivocal proteinuria accompanied these respective dose levels. With multiple 0.1 mg U kg^{-1} doses (every 6th day) α-NH_2 nitrogen, creatinine clearance and plasma urea nitrogen also began to change significantly in rats, whereas the other indicators tested were unaffected. At the 0.1 mg U kg^{-1} dose level in dogs, all indicators changed significantly after single intravenous doses or inhalation exposures, with some important variations. Such a pattern of response was seen at higher doses in rats ($\geq$1 mg U kg^{-1}).

Both species, also showed that some indicators, e.g., urinary α-NH_2 nitrogen (dogs and rats), creatine clearance (rats), returned to normal values within 7 days of single dosing, but others, e.g., N-acetyl-glucosaminidase (rats) and glomerular filtration rate (dogs) persisted for several weeks. Water consumption, and urinary volumes were also increased after UO_2F_2 administration and with the higher doses, these were elevated persistently in both species.

Collectively, these studies of renal function indicate multiple sites of uranium-induced dysfunction, i.e., involving both glomeruli and tubules; in the latter regard, responses are indicative of effects on the proximal, distal and collecting tubules and loops of Henle, and are in concert with histopathologic findings involving these structures which seem to be dose-related. While additional studies are needed, the use of urinary glucose for judging worker exposure, along with, or instead of, urinary protein, would appear advantageous.

(9) Synergism of HF and UO_2F_2: The possible synergism of UO_2F_2 and HF was investigated in both rats and dogs and the findings were similar. Both species appear to show slightly greater

urinary indicator responses, e.g., urinary volumes, glucosuria, following a combined inhalation exposure than when the same amount of UO_2F_2 was inhaled alone. This renal effect may be due to a weak potentiation of UO_2F_2 toxicity by HF in the combined exposure. The inadequate but available information on the nephrotoxicity of F^- suggest a simple combined action is also plausible, especially at the higher doses (>1 mg UO_2F_2 kg^{-1}) of both toxicants.

(10) The 30 μg l^{-1} Action Level: The NRC action level of 30 μg U l^{-1} of urine is derived from the steady state conditions predicted for the human kidney that were utilized in the "soluble" uranium MPC calculation. The NRC has utilized this action level only in uranium mills and certain U conversion facilities. No specific action level or guidance has been directed at UO_2F_2 or UF_6 per se or to U^{+6} generically. On the other hand, all systemic transport and urinary forms of uranium are believed to be identical i.e. exist only as UO_2^{++} and reversible UO_2^{++} complexes, hence renal-urinary uranium relationships are not dependent on the form of uranium exposure. Additionally, the action level would not seem to be applicable to single uranium intakes and this was confirmed by our UO_2F_2 studies in both species.

A single intake of 25 μg U kg^{-1} should result in a peak renal U concentration in man (~0.6 μg g^{-1}) which is believed to be below the injury threshold; nevertheless, this intake should also yield ~800 μg U l^{-1} urinary output during the first 24 hours post-exposure (0.64 x 1750 μg U). This output prediction is verifiable by the earlier intravenous studies in man at below nephrotoxic doses, <70 μg U kg^{-1}. A similar calculation based wholly upon these new UO_2F_2 studies in dogs would yield ~210 μg U l^{-1} urinary output during the first day for a comparable intake.

Using the new renal retention function of Stevens *et al.* ($\frac{0.693}{79\ day}$ = 0.009 day^{-1}) and a 0.6 μg g^{-1} steady state renal concentration as acceptable, only ~1 μg U l^{-1} would be excreted by man. This estimated urinary uranium output neglects any contribution from other deposits, e.g., skeletal uranium, which might double this value.

There are also minor problems in the action level concept associated with normal variations in body size and urinary output, but, clearly the major defect is in the unreliability of the 15 day renal retention half time and the 3 μg U g^{-1} renal concentration values. Consequently, we recommend both a 1 μg U l^{-1} Monday morning urinary excretion rate and a exposure-associated urinary output of 100 μg U l^{-1} during the first 24 hours post-exposure, as action levels. Less conservative output values (factor 5 greater) might be justified depending on the decision regarding the comparison of human susceptibility to uranium with that of the dog and rat: a mid-point decision provides such a factor.

II. INTRODUCTION

These studies of UF_6 toxicology, initiated in rats and dogs in 1978, were directed primarily at providing biological information for bioassay procedures of the U.S. Nuclear Regulatory Commission. It is well established that human exposures result from accidental releases of UF_6 vapor which spontaneously converts to UO_2F_2 and HF according to the reaction: $UF_6 + 2H_2O \rightarrow UO_2F_2 + 4HF$.

The extent to which this reaction proceeds depends mainly upon release rates, time, and the ambient relative humidity. Most UF_6 releases are promptly and completely converted to UO_2F_2 fume and HF gas. In major releases of UF_6, other airborne species of uranium possibly occur, but their conversion to UO_2F_2 is still highly probable within the nearly water-saturated air of human airways. While these statements about high release rates are somewhat conjectural, the simplification of all UF_6 exposures from a toxicological perspective to that of a UO_2F_2 aerosol-HF gas mixture seems justified. The major effect of high UF_6 release rates would be on the particle size of the UO_2F_2 aerosol and the concentration of the highly reactive HF gas and their respective hydration states. Because of the extreme water solubility of both UO_2F_2 and HF, their prompt and complete dissolution is assumed at all sites of respiratory deposition. The particle size of the aerosol and concentration of the gas (and possible association of HF with particles) will affect their respective respiratory deposition patterns qualitatively and quantitatively but probably will not affect retention rates or extent at any given deposition site.

While the foregoing viewpoints are not completely established, they were expected to prevail, and our subsequent studies have generally supported them. This circumstance is fortuitous, for we can see the possibility of comparing biological and toxicological responses among species by utilizing absorbed dose. If the chemical toxicity of U^{+6} in the kidney is limiting, as it seems to be, then the sites of uranium absorption become less significant, except to the extent that they affect the degree of absorption. In this latter respect, differences in absorption between uranium deposited in the nasal airways, translocated uranium in the gastrointestinal tract, and uranium deposited in the lungs were expected to be manifest and they were found. Thus, species comparisons were destined to be affected by quantitative differences due to the initial pattern of UO_2F_2 intake as well as by intrinsic differences in the species themselves.

A major question to be answered was: Can the absorbed dose of uranyl fluoride be correlated with renal injury or urinary uranium excretion rate This correlation is implied indirectly by the ICRP excretion equation for uranyl uranium. Inasmuch as uranium-induced renal injury in uranium workers is presently assessed by action levels and urinary indicators, e.g., proteinuria, the possibility of correlating urinary excretion of uranium, absorbed dose, etc., with renal injury, depends critically on the reliability and sensitivity

of the indicator methods, so these too became a major part of the experimental studies.

These dog and rat studies with the hydrolysis products of UF_6 were accomplished in two phases. The first of these was reported in NUREG/CR-1045 (Morrow et al., 1980) for the experimental period June 1978 - July 1979, whereas, this report pertains principally to the second phase studies: July 1979 - Oct. 1981. However, in all important respects the new data are amalgamated with and compared to those of the first phase and presented in a final, summary form. This integration of results was adopted since the later studies were intended to extend both the dose and time bases of the distribution and excretion data while repeating some of the former exposure conditions. Additionally, new studies were included to clarify specific issues, e.g., does exposure to UO_2F_2 in the presence of a stoichiometric amount of HF differ toxicologically from the same concentration of UO_2F_2 Does a repeated administration of UO_2F_2 lead to different excretion rates or modify the biochemical indices of renal injury To assist in obtaining information on such questions, we again utilized intravenously-administered UO_2F_2 since we found that this provided similar excretion data to those from inhalation exposures, but with more precisely controlled dose administrations. There were some distinctions in U^{+6} distribution found between the two routes of administration, however, and these will be cited. Also both intravenous and intratracheal studies were undertaken in rats to facilitate the acquisition of dose-response data in that specie.

In all major regards the completed study was successful in achieving the primary experimental objectives (See Section IV).

One peer-reviewed report has been prepared covering the exposure, distribution and excretion of uranium in dogs following aerosol exposures and intravenous injections of UO_2F_2 (Morrow et al., 1982); these topics were also the basis of the 1981 meeting presentation by the same authors (Morrow et al., 1981). Biochemical indicators in the rat of uranyl fluoride toxicity following intratracheal administration have also been reported (Scott et al., 1980). Additional reports are contemplated.

III. SELECTED HISTORICAL REVIEW

The types of animal studies performed at Rochester in the 1940's and 1950's have been detailed in the four volume monograph of Voegtlin and Hodge (1949-1953) and summarized more recently by Yuile (1973). Also Durbin and Wrenn (1975) prepared an extensive review of the animal data on uranium. The human data on uranium metabolism and toxicity have been summarized by Hursh and Spoor (1973), Adams and Spoor (1974) and by Boback (1975).

In relation to the study being reported, the cogent literature is restricted to U^{+6} compounds administered parenterally or inhaled and includes a few new studies of specific relevance, e.g., Neton *et al.* (1979) and Stevens *et al.* (1980).

Perhaps the most appropriate findings from the animal studies are:

(1) The dog is more susceptible to U^{+6} effects than the rat (Yuile, 1973).

(2) UO_2F_2 is the most toxic of uranyl compounds in acute studies in rats e.g. 2X more toxic than uranyl nitrate, and in the long-term feeding studies, it was 4X to 6X more toxic than uranyl nitrate (Yuile, 1973).

(3) Urinary catalase was probably the most sensitive indicator found for U-induced renal injury with as little as 0.01 mg kg^{-1} of U^{+6} producing catalasuria in 2 to 4 days in rabbits. In the early literature, catalase excretion was not believed to increase in man, however (Yuile, 1973).

(4) Tolerance to uranium toxicity, induced by prior exposure to U^{+6}, was judged by changed mortality at dose levels usually producing lethal renal damage. Rats required a dose $\geq$0.1 mg kg^{-1} U^{+6} to affect tolerance and, depending upon the inducing dose, the tolerance persisted for between 1.5 and 6 months. Tolerance was reported to be associated with tubular regeneration and elevated renal citric acid elimination (Yuile, 1973; Haven and Randall, 1947).

The human data are especially pertinent to the following points:

(1) The older studies underscore the controversial question: is U^{+6} a specific poison for the distal third of the proximal tubule There are data indicating it has a glomerular action of importance, but its comparative timing, sensitivity or consequence with regard to tubular necrosis, remains unclear (Hodge, 1973).

(2) Intravenous doses of uranyl nitrate between 70.9 and 100 μg U kg^{-1} produced transient renal injury in the Rochester and Boston studies, whereas 6-70 μg kg^{-1} doses did not (Hursh and Spoor, 1973). Doses within this non-toxic range nevertheless resulted in urinary uranium outputs between 10^2 and 10^3 μg U l^{-1} for one

or more days (Bassett et al., 1948), clearly exceeding the NRC action level of 30 μg $U\ l^{-1}$ (NRC, 1978).

(3) Intravenously administered uranyl nitrate (<165 μg kg^{-1}) resulted in 57-83 percent uranium elimination in the 1st 24 hours, and 70-90 percent in 5 days, exclusively by the urine. Subjects receiving doses ≥283 μg kg^{-1} or manifesting pre-existing renal dysfunction generally showed reduced U^{+6} excretion over the same period (Basset et al.; 1948; Struxness et al., 1956 and Terepka et al., 1964).

(4) Tissue data from subjects receiving <165 μg kg^{-1} i.v. (Boston study), yielded bone levels at 2.5, 18 and 74 days of 10, 4.9 and 1.4 percent of the administered dose, respectively, whereas the kidney contained 16.6, 7.2 and 0.7 percent at the same times (Hursh and Spoor, 1973). Retention functions for uranium in the human kidneys and skeleton have taken several forms. Using two-term exponental functions (t = days), they are: $R_K(t) = 0.16 \exp(-0.693\ t/12) + 0.01 \exp(-0.693\ t/1100)$ and $R_S(t) = 0.10 \exp(-0.693\ t/15) + 0.01 \exp.(-0.693\ t/3200)$, respectively, indicating that both organs have a small, but protracted uranium retention (Adams and Spoor, 1974).

(5) The maximum permissible renal concentration of 3 μg U g^{-1} is based largely on the animal studies at Rochester. The 5×10^{-3} μCi MPBB and a f_2 value for kidney of 6.5 percent, also indicated that ~3 μg U g^{-1} was an acceptable radiation level for natural uranium in that organ. Assumptions included a 15 day effective half-life for U in the kidney and a 300 gram kidney mass for man (Spoor and Hursh, 1973).

(6) The International Commission on Radiological Protection (ICRP, 1968) uses a modified form of the Lippman et al. (1962) equation for the daily rate of uranium excretion (Y_u) in the urine:

$$Y_u = 0.8 \text{ (1st 24 hours)}$$

$$Y_u\ (t) = 0.2t^{-1.5} \text{ (at } t > 1 \text{ day)}$$

The ICRP equation considers the fraction excreted per day in terms of total urinary U excretion.

(7) Accidently-exposed uranium workers (UF_6) with intakes estimated to be around 0.1 to 0.2 mg kg^{-1} (5 to 12 mg U) showed no evidence of renal injury on the basis of protein, sugar, pH, specific gravity and microscopic analysis of their urines (Boback, 1975).

IV. RESEARCH OBJECTIVES

The experimental plan was designed to examine primarily the relationships of inhaled UO_2F_2 and HF (hydrolysis products of UF_6) and (a) the resulting uranium burden in the lung, kidneys, and whole-body, and (b) the subsequent uranium elimination patterns. Two species, the rat and dog, were selected to reveal or verify any species distinctions. A range of uranium doses was planned to establish the appropriate level for renal injury using the most sensitive indicators of injury at our disposal. Studies using doses above and below this injury threshold were of interest in order to determine if changes in uranium retention or excretion would occur in relation to dose. To facilitate specific quantitative exposures of animals to UO_2F_2 for the dose-effect experiments, intravenous and intratracheally-administered uranium were used as parallel approaches to the inhalation exposure.

Experiments were also planned on closely related problems: specifically, to examine the possibility of finding and applying a more sensitive renal injury indicator; to determine if stoichiometric amounts of HF and UO_2F_2 act synergistically or cause U^{+6} to behave differently *in vivo* than when administered as UO_2F_2 alone; to investigate possible distinctions between naive and repeatedly-exposed subjects; and to examine the related "tolerance" effect.

In all of the foregoing areas, the experimental design provided a basis for comparing two laboratory species and each with man, and for characterizing, metabolically, UO_2F_2 in relation to other important U^{+6} compounds. Through these studies and comparisons, the usefulness of extant NRC bioassay procedures are to be judged.

V. MATERIALS AND METHODS

1. Uranium Compounds

Both UO_2F_2 and UF_6 were procured in two forms: the first form was prepared from natural uranium and was used for pilot studies; the second form was synthesized from enriched uranium having the following isotopic composition: U-234: 0.74 percent; U-235: 93.14 percent; U-236: 0.08 percent and U-238: 6.04 percent*. The enriched uranium had an alpha-emitter specific activity of 4.9×10^{-5} Ci g^{-1} and this was largely attributable to the U-234 content. The enriched uranium, by virtue of its U-235 content was also amenable to gamma counting of the 95, 143, 165, 185 and 200 keV photons, especially the latter two which constitute 66 percent of the gamma photons.

When UF_6 is discharged into water or a relatively large volume of ambient air, the UO_2F_2 formed, either by drying the solution (e.g., vacuum distillation) or spontaneously as a fume, exists as the dihydrate (Pickrell, 1980). No appreciable amounts of other oxyfluorides or free UF_6 are detectable under these conditions. All references in this report to particulate UO_2F_2, therefore, should be understood to mean $UO_2F_2.2H_2O$.

2. Analytical Methods for Uranium

Three analytical methods for uranium were employed: (1) liquid alpha scintillation counting, using a dual detector pulse shape discrimination system (Sperr et al., 1974 and Horrocks, 1974), (2) gamma counting using a 3" x 3" NaI(Tl) scintillation counter for dog tissues and excreta, a pair of 2" x 4" NaI(Tl) scintillation counters (within a steel chamber) as a thoracic or whole-body counter for dogs, and for counting rodents (rats) a 2" x 6" sodium iodide well counter coupled to a Canberra 1431 single channel analyzer (Figure 1) and finally (3) a colorimetric method of Baumann, 1977, was used to measure uranium content of filter paper dust samples, particle-size stages of a cascade impactor and of other non-biologic samples. The colorimetric method was calibrated by a standardized uranium solution (Anderson Laboratories I.C. 81610).

For alpha counting, biological materials were dried under heat lamps and then ashed at 550°C in platinum dishes before dissolution in 1N nitric acid. If a clear solution did not result, the acidic solution was heated to NO_x fumes, treated with 30 percent hydrogen peroxide, redissolved in 1N nitric acid, and adjusted to a constant

* Provided by Goodyear Atomic Laboratories, Dayton, Ohio, as Lots 952346 and 972275. Isotopic composition determination by mass spectrography using an electron bombardment source with accuracy of approximately ± 0.01 percent (Personal communication, Mr. Trivisanno, Goodyear Atomic Labs).

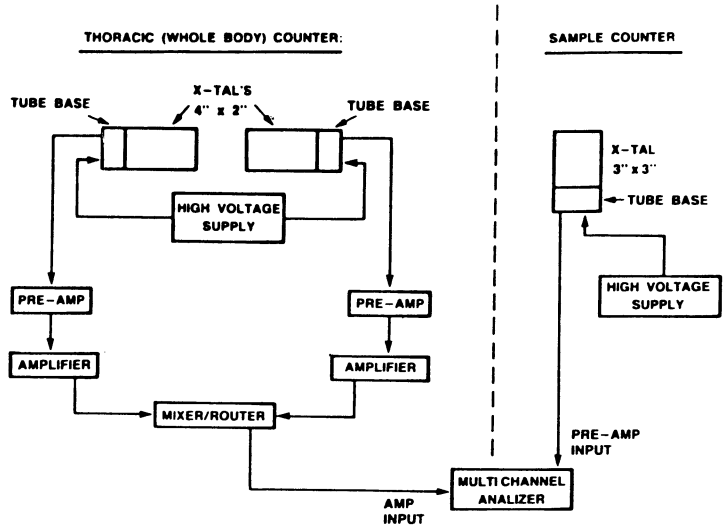
Figure 1. Radioactivity Counting Systems

(A) U-235 Gamma Counting System: Dog Study.
The sample counter (right) utilizes an Ortec Model 446 High Voltage Supply. The detector output is directly coupled to the Pre-Amplifier Input of the Tracor-Northern, Econ II Multichannel Analyzer (MCA). The dog counter (left) uses paired Tennelec Model 155A Pre-Amplifiers and Ortec Model 435A Amplifiers with a single Tracor-Northern Model NS-459 Mixer-Router coupled to the amplifier input of the Model 710 MCA. The data output is recorded on a Hewlett Packard Model 2 FRA X-Y Recorder and Model 3320-3JC Teletype.

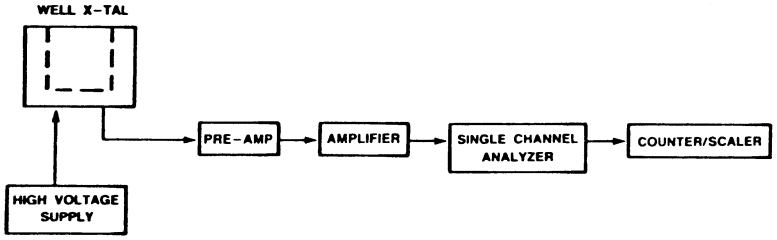
(B) U-235 Gamma Counting System: Rodent Study.
A 3" diameter by 5" deep well in a 5" x 6" sodium iodide crystal detector (Harshaw 20 MBW 24/5A) was used for gamma counting a variety of biological specimens. The system consists of a Canberra Model 805 Pre-Amplifier, a Model 816 Amplifier, a Model 1431 Single Channel Analyzer and Model 1491 Counter Scaler. High voltage was supplied by a Hewlett-Packard Model 6515A Power Supply.

(C) Alpha Counting.
The system utilizes RC4500/V4 photomultipliers whose outputs are directed to paired Canberra Model 2160 Pulse Shape Discriminators and Model 1428 Constant Fraction Discriminators. The Time Analyzers are Canberra Model 1443A. The remainder of the system consists of a Canberra Model 1776 Dual Counter-Timer, Model 1446 Universal Coincidence, 2 Model 2000 BIN Supplies and a Series 30 Multi-Channel Analyzer. The Delay Amplifier is a Tennelec Model TC-215 and the output recorder, a Newport Model 810 Printer.

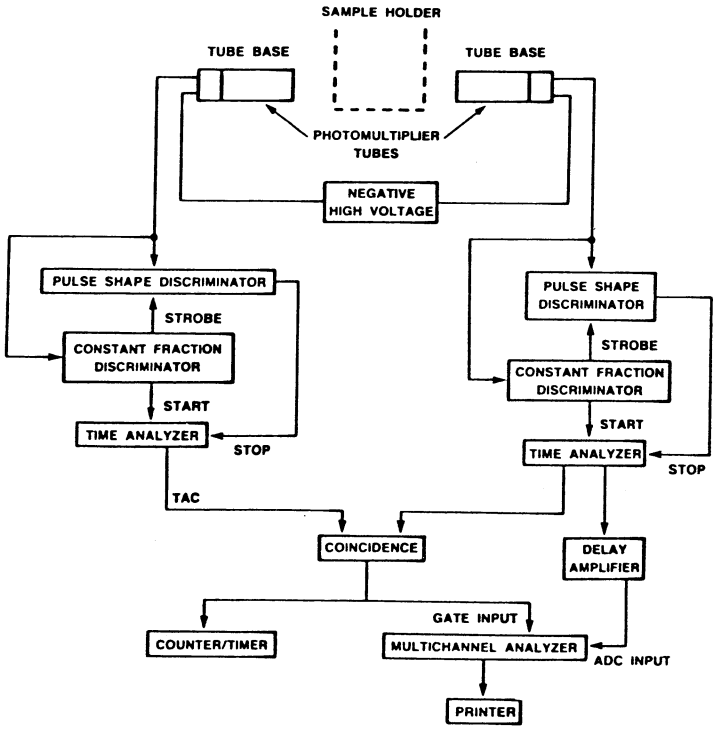
A U-235 COUNTING SYSTEM FOR DOG STUDIES



B U-235 COUNTING SYSTEM FOR RODENT STUDIES



C ALPHA COUNTING - LIQUID SCINTILLATION SYSTEM



volume. Dog fecal sample digests contained insoluble silica and silicates: these entrained negligible uranium, and so were filtered. Aliquots (0.3 ml) of the acid digests were added to 6 ml of a commercially-available scintillation cocktail (Beckman Ready Solv-HP) to which 0.3 ml of a stabilizer (Beckman Biosolv BBS-3) had been added. All samples were prepared in duplicate and one sample of each pair was spiked with a 10 µl aliquot of a standardized uranium solution in order to correct for quenching and other effects. All samples, standards and blanks were counted with approximately 95 percent efficiency in a dark, refrigerated (5°C) liquid scintillation counter (Figure 1-C) for a minimum of 10 minutes, using 7 ml foil-lined, screw-capped borosilicate vials. With the usual counting protocol, the practical detection limit was 0.1 µg U. Although substantially improved counting conditions were possible by eliminating nitrate, degassing with argon, etc., these additional steps were not time and cost efficient in relation to the simpler internal standard method and the analytical sensitivity achieved.

Gamma counting of tissue and excreta samples utilized calibration curves and standardized counting configurations so that absorption and geometric factors were compensable. Dog counting entailed placing the anesthetized animal in a special holder which could then be rolled into a lead-filled steel chamber. An Alderson dog phantom containing 16 standarized ^{235}U sources in the lung fields was used to help quantify the *in vivo* counting of ^{235}U. Gamma counting times varied from 600 to 2000 seconds. Backgrounds of all dogs were taken prior to exposure and were used to correct the body counts. The counting efficiency of the various gamma counters for the 186 kV gamma photon of ^{235}U were as follows: rodent well counter ~62 percent; dog sample counter ~15 percent and the dog whole-body counter ~2 percent, respectively.

3. Animals

Purebred beagle dogs were procured from Marshall Farms (North Rose, NY) or Buckshire Corporation (Perkasie, PA). The young adult female dogs of 7.2 kg mean body weight (range 6-10 kg) were housed in individual metabolic units (Hazelton Model RD 40) after a quarantine period of at least 30 days during which a full clinical evaluation was made for each animal by the University's Laboratory of Animal Medicine and control data were acquired on blood and urinary parameters.

The rodents used in these studies were male rats (initially weighing between 200 and 300 g) of the Long-Evans, LE, (Charles River Breeding Laboratory Incorporated, 251 Balardvale Street, Wilmington, MA) or Sprague-Dawley, SD, (Blue Spruce Farms, Altamount, NY) strain. These animals were maintained on a diet of tap water and Purina Rat Chow and were housed in special metabolism units serviced only by research personnel. Prior to treatment, the urinary and fecal pattern of excretion was established for selected animals and 24 hour urine collections were reserved for control purposes. Also,

a sample of the blood (0.1 ml) was taken for control plasma urea nitrogen (PUN) determination.

4. Exposure Methods

A. Dogs (Morrow et al.)

For the intravenous administration of UO_2F_2, an appropriate aliquot (< 1 ml) of 1 percent (w/v) UO_2F_2 solution in water was diluted to 10 ml with isotonic saline and slowly injected (~3 ml min^{-1}) into an antecubital vein of the anesthetized animal (secobarbital sodium, ~22 mg kg^{-1}, intravenously (I.V.)).

For inhalation exposures, dogs were anesthetized with I.V. sodium pentobarbital (~26 mg kg^{-1}) and placed into individual body plethysmographs with their heads exteriorized through a neck seal. Volume meters were connected to the plethysmograph and the dogs were intubated with a cuffed endotracheal tube (American Hi-Lo 9 mm I.D.). The endotracheal tube in turn was connected to a Rudolph valve so that inspiratory and expiratory volumes could be separated and analyzed. Two such dog units were connected to a chamber (30 liter) into which the aerosol was delivered (Morrow et al., 1980). The exposures were varied from 30 minutes to about 2.5 hours. The UO_2F_2 aerosols were dispersed from a DeVilbis 645 Nebulizer modified so that a Braun 1830 Infusion pump delivered ~0.3 ml min^{-1} to the nebulizer via an air-driven reservoir of UO_2F_2 solution which kept the nebulizer output reasonably constant. For inhalation exposures involving HF, enriched UF_6 was dispersed into a chamber used for rodent studies (Sandia Nose-only Unit). Refer to Section 4.B. Rats. In place of a rat holder being connected to the chamber, the dog's endotracheal tube was connected using a stopper. Dogs were exposed to UO_2F_2 in the presence of HF at UO_2F_2 concentrations of about 250-720 mg m^{-3} for 30 minutes to 1 hr using the same plethysmographic arrangement.

B. Rats (Leach and Gelein)

For the inhalation experiments with rodents, vaporized UF_6 was metered from a heated (about 57°C) gas cylinder into the inlet air system of a "nose-only" exposure unit that was described in detail in our earlier report (NUREG/CR-1045, Morrow et al., 1980). Before introduction into the exposure chamber, the concentrated vapor was diluted with clean, compressed air maintained at a relative humidity of approximately 50 percent, thus insuring complete hydrolysis of the UF_6.

Groups of six to 37 rats were exposed simultaneously, while each rat was maintained unanesthetized in a special restraining tube connected to the exposure chamber. This arrangement minimized deposition of the UO_2F_2 aerosol on the fur of the animals since only a small portion of the nose contacted the test atmosphere. Immediately after exposure, the rats were removed from the restraining tubes and their heads were washed with aqueous detergent to remove any deposited UO_2F_2.

In the intravenous injection studies with rats 0.1, 1.0 or 5 mg U (as UO_2F_2) kg^{-1} body weight was slowly injected into each animal by way of a tail vein. The aqueous U solutions were administered in volumes of approximately 0.2 ml/100 g of body weight, and saline solutions of the same volume were given for control purposes.

C. Rats: Intratracheal (Smith, et al.)

This study was undertaken to investigate how well animals with a pre-existing renal burden of 3μg U g^{-1} of renal tissue would be able to cope with a subsequent uranium exposure. The figure of 3 μg was chosen inasmuch as the Nuclear Regulatory Commission considers that individuals with less than this level of uranium in the kidney may be safely employed in the uranium industry. The problem was approached in terms of episodic exposure followed by a decline in renal burden, rather than from gradual buildup of uranium in the kidney. The experimental model was as follows: Rats were given UO_2F_2 by intratracheal instillation at a dosage such that a renal burden of about 3 μg U g^{-1} tissue was present approximately 30 days later; 60 days after the initial instillation, the same dosage of UO_2F_2 was administered again. Throughout the entire study period the response of the animals was assessed in biochemical and histopathological terms.

Details of the intratracheal instillation of UO_2F_2 have been described earlier (Morrow et al., 1980). The procedure is a modification of that described by Brain et al. (1975). Male Sprague-Dawley rats were anesthetized with 0.6-0.7 ml of sodium pentobarbital solution (65 mg ml^{-1}) given intraperitoneally, and then fastened on a slanted board. A small area of the trachea around the larynx was exposed surgically. UO_2F_2 solutions were delivered to the lungs with an 18 gauge needle attached to a 1 ml syringe. The needle was inserted into the trachea between the cartilagenous rings and then inserted a further 1/2 inch distally into the trachea. Each animal received 0.10 ml of UO_2F_2 solution per 100 g of body weight, the solution being instilled during an inspiration. The animal was kept upright on the board until regular breathing resumed; the chest was gently massaged if difficulty in breathing was encountered. The rat was removed from the board, the incision closed with autoclips and the animal placed face down, head up on an inclined surface to recover from the anesthetic. Upon recovery, he was then removed to an individual metabolism cage.

5. Air Sampling

During all of the exposures involving hydrolyzed UF_6, the UO_2F_2 aerosol concentration and aerodynamic particle size was determined by periodically sampling the chamber atmosphere with a filter paper dust sampler, containing a membrane filter (Type AA Millipore) and an eight-stage cascade impactor (Mercer et al., 1970). The aerosol samples were analyzed for U by either alpha counting or by a colorimetric method (Baumann, 1977). Hydrogen

fluoride levels in the chamber were measured using two in-line bubblers (installed behind the filter paper dust sampler) each containing 5 ml of an aqueous buffer (TISAB Orion No. 94-09-09A); the absorbed fluoride was determined with a fluoride ion specific electrode (Orion 96-09 Combination Electrode) in conjunction with a Orion Ionanalyzer Digital pH Meter (Model 801) with a practical detection limit of 10 ng F/ml.

6. Excreta Collections

Using the metabolic units, daily or more frequent collections of dog urine and feces were made by technical personnel. The cage floor and drain were cleaned at each collection and the cage wipes were also analyzed for U. During the first phase of these studies, most of the dogs were catheterized and urine collections were made hourly after UO_2F_2 administration. Special tests in dogs, e.g., inulin clearance, were also undertaken with catheterized urine collections and concomitant venous blood samples. For more protracted studies, excretion samples were obtained mainly from the metabolism cages.

In all of the experiments with rats (inhalation, intratracheal and intravenous) the urine and feces from each animal were collected daily and subsequently measured for U content by alpha or gamma counting. Selected samples of urine and blood were used to evaluate the usefulness of indicators of renal injury.

7. Indicators of Renal Injury

A considerable emphasis was placed on this investigative area since it was of special significance to the establishment of a renal injury dose threshold, and because we wished to identify the most sensitive and useful indicator(s) for both naive and pre-exposed animals. The latter objective did not ignore the original studies and their findings (Berke, et al., 1953), but we assumed technological advancements might produce new findings in these regards. Also some of the indicator methods we planned to employ were not studied adequately, or at all, in the previous work.

For the dog studies, inulin clearance measurements (glomerular filtration rate, GFR) were performed while the dogs were catheterized by using Methoxy-^{14}C- Inulin (New England Nuclear 692). Each dog served as his own control. Samples of blood and urine were mixed with a tissue solubilizer (Amersham NCS-190610) and allowed to stand overnight. A scintillation mixture (Research Products International) was added and the samples were counted in the dark and cold using a Beckman LS-50 Liquid Scintillation Counting System.

In both rats and dogs, plasma urea nitrogen was determined (Chaney and Marbach, 1962) as well as urinary citrate (Lowry and Passanneau, 1972), urinary protein (Lowry et al., 1951), plasma and urine creatinine (Heinegard and Tiderstrom, 1973), urinary glucose (Lowry and Passanneau, 1972), urinary alpha-amino acid nitrogen (Lorentz

and Flatter, 1974), urinary phosphate (Chen et al., 1956) and urinary N-acetyl-beta-glucosaminidase (Lockwood and Bosmann, 1979). Most of the foregoing tests were applied routinely to 24-hour urine collections or periodic blood samples, as appropriate. Additional information on daily urine output, food and water intake, and body weight was recorded.

In the initial phase of the study, a special fibrin antibody assay developed by Spar et al. (1959) was applied to animals prior to sacrifice. The ^{125}I labelled antibody was expected to concentrate at sites of injury, but significant localization of radio-iodine did not occur in the kidneys at times when other criteria indicated renal injury existed, so this effort was not continued.

8. Necropsy Procedures

Dogs were given an overdose of intravenous pentobarbital at different times, from a few hours to 19 days postexposure, and tissues were immediately taken for analyses. For U determinations, bone and most soft tissues were sampled initially, but this was later reduced to bone, liver, spleen, lungs and kidney on a routine basis because insignificant U levels prevailed in all other tissues. The entire organs were analyzed except for liver and bone (rib and leg) where samples were limited to about 30 grams each. Specimens of lung and kidney tissues were routinely prepared for histopathology (hematoxolin-eosin staining of ~10 μm thick paraffin embedded sections) and to a lesser extent for autoradiography.

Each rat was necropsied upon death or at sacrifice, and major organs including bone and skin were assayed for radioactivity. Selected tissues were also prepared for histopathology. The sum of the U contents of the dissected parts of the rat* plus that found in the urine and feces was generally used as the value for the initial body burden or inhaled dose. During dissection, the skin of the head of each rat was removed and counted for radioactivity. These values, although usually low because of previous washing, were not included in the figures for inhaled dose and were recorded as external contamination. The reconstituted body burden proved to be a more reliable figure than that obtained from whole-body counting of the live animal because of counting geometry problems associated with the well counter and the possibility of aerosol deposition on the fur of the head and external nose of the test animal.

*Lungs, liver, spleen, carcass, gastrointestinal tract, testes, blood, urinary bladder, heart, trachea and esophagus were generally assayed for U.

VI. RESULTS

1. Dog Studies (Morrow, Beiter, Gelein and Yuile)

A. Exposures

For the dog studies the UO_2F_2 aerosol mass concentrations were varied between 100 and 500 mg m^{-3} and the exposure times between 0.5 and 2.5 hours. In Table 1, a summary of the nineteen studies is given which indicates that the inhalation doses ranged from 0.11 to 1.46 mg U kg^{-1} and the postexposure period for follow-up measurements ranged from 6 hrs to 19 days. Of the 16 dogs studied, 3 were not sacrificed at the end of their postexposure period, but re-exposed at a later date. These animals were used for their excretion data: their urinary biochemistry had reverted to normal before re-exposure. Their second exposure was consequently designed to contribute to our study of naive versus previously-exposed animals.

In two of the nineteen studies, HF1 and HF2, dogs were exposed in a chamber wherein UF_6 decomposed spontaneously to stochiometric levels of UO_2F_2 and HF gas. For example, if the UO_2F_2 exposure concentration was 310 mg m^{-3}, then in theory 80 mg m^{-3} of HF gas should exist. However, in four determinations of the HF levels in the rodent exposure unit, the levels were typically about one-half of theoretical. Presumably, this loss was due partly to HF reactivity with chamber surfaces, though some HF may have become incorporated into the UO_2F_2 particles (See VII. Discussion). Although the UO_2F_2-HF dogs were exposed to a slightly different aerosol (See Table 1), the distribution and excretion data were indistinguishable from the UO_2F_2 (alone) data in that the cumulative urinary uranium excretion results were within $\pm$ 1 percent of the 24, 72 and 144 hour means in Table 2 and the tissue levels were within 0.5 percent of the absorbed dose values from dogs sacrificed after comparable post exposure times; consequently, all of the inhalation study data were pooled.

Because of assorted technical problems, deposition measurements were made reliably in only 12 of the 19 UO_2F_2 aerosol studies. The mean total lung deposition was 42 percent (30-58 percent range) of that inhaled. Thus, for uniformity, the total recovered dose is used as the absorbed dose in all dog studies. Where comparisons were possible, all of the deposited and absorbed doses agreed to within $\pm$ 10 percent.

Five dogs were investigated in nine studies using intravenous UO_2F_2 administration. Two of these were used for a single re-exposure after their urinary biochemistries were within the normal range. Thus, intravenous uranium doses varied from 0.01 to 1.95 mg kg^{-1} and are expressed as injected doses. These corresponded to 100 $\pm$ 3 percent of the recovered doses and were studied over postexposure periods ranging from 26 hours to 14 days. Studies V-1/V-2 uniquely involved the same dog which was exposed

Table 1: Exposure protocols

Inhalation Studies

UO_2F_2 aerosol (mean) MMAD 1.04 μm σ_g 1.59

Study No.	Dose (mg U kg^{-1})	Postexposure (days)‡
I-1	0.11	6
I-2	0.14	7
I-3* (7)	0.15	6
I-4	0.22	6
I-5	0.25	7
I-6	0.50	8
I-7* (75)	0.58	14
I-8	0.62	8
I-9	0.67	1
I-10	0.68	19
I-11	0.68	0.25
I-12	0.70	19
I-13	0.73	3
I-14	0.75	1.2
I-15	0.78	14
I-16	0.92	3
I-17	1.46	1.1
HF and UO_2F_2 aerosol (mean) MMAD 1.07 σ_g 1.73		
HF-1	0.30	6
HF-2* (270)	0.55	16

Table 1: Exposure protocols (cont.)

Intravenous Studies

UO_2F_2 solution ~0.01 % (w/v) in isotonic saline

Study No.	Dose (mg U kg^{-1})	Post Adm.‡ (days)
V-1	0.01†	6
V-2* (6)	0.01**	5X6
V-3	0.12	6
V-4	0.43	6
V-5	0.44	14
V-6* (14)	0.44	14
V-7* (6)	0.45	6
V-8	0.88	2.1
V-9	1.95	1.1

* Dog from prior exposure. Number in parentheses indicates days separating end of first postexposure period and re-exposure.

† First of 5 identical I.V. doses.

** Mean of 5 identical I.V. doses 6 days apart.

‡ Time to sacrifice, except for re-exposed dogs (*).

more frequently than twice. This dog received 5 identical 0.01 mg U kg^{-1} intravenous doses during 24 days; after an additional 6 days, the dog was given a single higher dose (0.56 mg U Kg^{-1}) and followed another 6 days as study V-7.

B. Uranium Excretion

A weak correlation between dose (mg U kg^{-1} injected) and 24 hour urinary uranium excretion, was noted in some of the human studies (Hursh and Spoor, 1973). Our final canine data revealed no such trend. The mean urinary uranium excretion from all dog studies was 63 (0.96 x 66) percent (S.D. 10.2 percent) in the first 24 hour urine, whereas approximately 2.6 (0.04 x 66) percent of the absorbed dose was found in the feces (Table 2).

The subsequent removal of the uranium in the urine, i.e., the fraction of the absorbed or injected dose hr^{-1} followed the power function: $Yu = .006t^{-1.5}$ very closely (Figure 2). Yu is equivalent to $0.14t^{-1.5}$ when t is expressed in days. If the total urinary uranium excretion is taken as the cumulative 19 day (456 hours) value in Table 2 (mean of 2 studies) then the first 24 hour-excretion, expressed as a fraction of the total urinary excretion, equals $\frac{.96 \times .66}{.935 \times .82} = 0.82$; the subsequent daily excretion rate becomes $Yu(t) = 0.18\ t^{-1.5}$. Thus, both expressions closely agree with those of the ICRP for U^{+6} compounds (ICRP, 1968).

In Table 2 and Figure 3, the excretion and distribution data from all 19 inhalation studies are summarized. The 24, 72 and 144 hour, mean cumulative excretion values are based on 10 or more animals; consequently standard errors were computed. For the remaining times, only from 2 to 5 values were averaged, due to attrition of dogs by serial sacrifice. Some dogs showed insignificant uranium levels in the excreta at 168, 336 and 456 hours post exposure.

C. Uranium Retention

The Table 2 results indicate that the whole body retention function $R_{(WB)} = 0.67e^{-3.4t} + 0.18e^{-0.49t} + 0.15e^{-0.043t}$ applies satisfactorily to the 19 day data where t is expressed in days. These three retention rates are represented by 0.2, 1.4 and 16 day half-times, respectively.

The kidney data show a build-up before 6 hours (Figure 3) and then a steady decline with the 24 hr to 456 hr data giving a 9.3 day half-time (R^2=0.97)*. These early measurements appear to fit the initial values in the two year data of Stevens et al. for

*R^2 is the coefficient of determination for the regression analysis performed on the clearance data (exponential fit).

Table 2: Uranium disposition following inhalation exposure (19 studies)

Time after Exposure (Hours)	Percent of Absorbed Dose						
	6	24	72	144	168	336	456
Mean Cumulative Excretion	28	66	82	87	89	92	93.5
(Standard Error)	-	(3.6)	(1.5)	(1.8)	-	-	-
% in urine	100	96	90	86	86	84	82
% in feces	0	4	10	14	14	16	18
Mean Body Burden	72	34	28	13	11	8	6.5
Kidneys	44	16	13	9	8	5.5	4.0
Skeleton*	5	7	7	3.5	3	2.5	2.5
Lungs	8	4	2	-	-	-	-
Liver	-	1	1	-	-	-	-
Other	15	6	5	0.5	-	-	-

* wt assumed equal to 0.15 body wt.

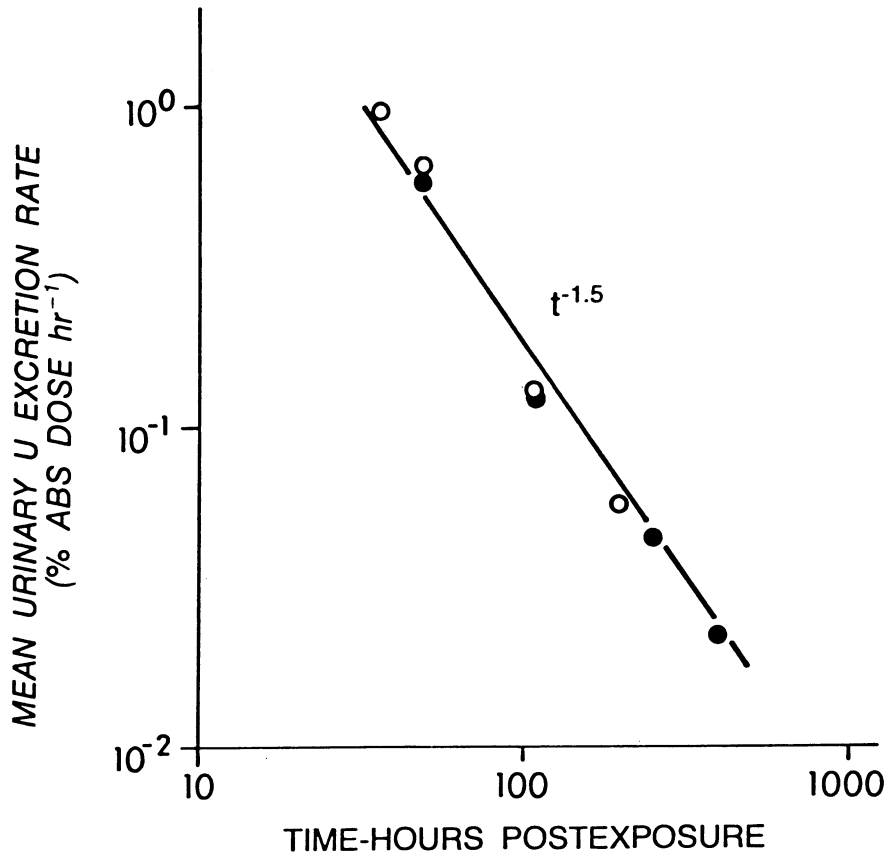


Figure 2. U^{+6} Excretion Rate.
Both inhalation (o) and intravenous (•) studies were utilized to obtain these average excretion rate values. These rates closely follow the power function described.

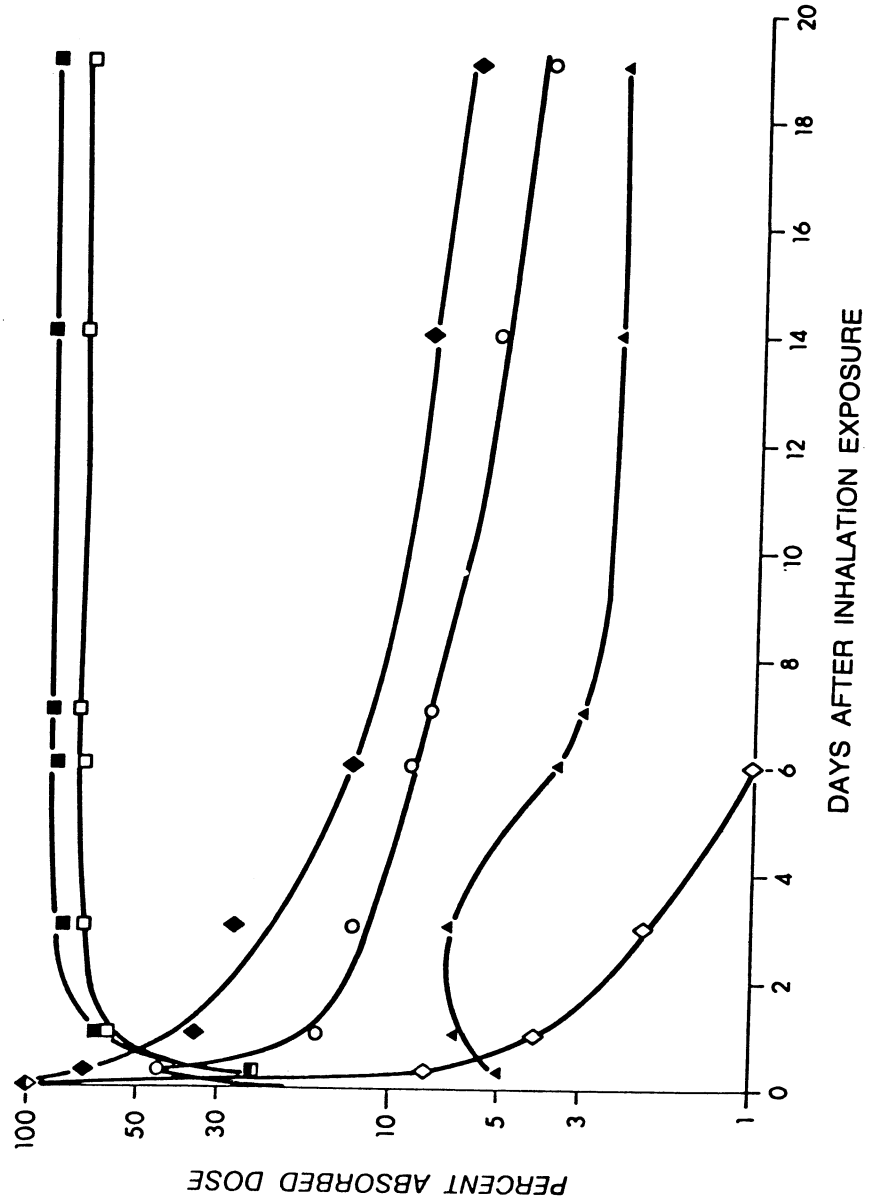


Figure 3. Inhalation Studies: Distribution and Excretion. The U^{+6} excretion (■) was used to compute the whole body retention (◆), i.e., 1 - $\sum$ excr. Urinary U^{+6} elimination (□) is also plotted as a cumulative curve. The kidney (○), estimated bone (▲), and lung (◇) contents are also depicted.

intravenous uranyl citrate in dogs which, when treated as a single exponential, yielded a 79.5 day half-time for uranium in the kidneys.

The skeletal data for UO_2F_2 dogs were estimated because it was necessary to factor a measured concentration of uranium in bone by the body weight, assuming the skeletal weight was 15 percent thereof. Also, there are insufficient UO_2F_2 data to obtain a useful retention function for uranium in bone.

In earlier reports (Morrow et al., 1972, 1980), we described the difficulties in interpreting the chest or thoracic gamma activity from inhaled uranium. Immediately after each aerosol exposure, gamma counting was started and it was determined that the average initial thoracic measurement was only 69 percent of the administered (recovered) dose (range 44-82 percent). When each dog was necropsied, the thoracic area was recounted with the lungs removed and even as soon as 12 hours postexposure, a significant fraction of the thoracic ^{235}U activity was found attributable to extrapulmonary uranium retention; hence, a second correction was required. A typical, reconstructed lung retention curve showing the magnitude of these corrections is given in Figure 4.

The uranium retention equation for the lungs (0-6 days) was computed to be:

$$R_{LUNG} = 0.89e^{-1.95t} + 0.07e^{-0.800t} + 0.04^{-0.015t}$$

where t is expressed in hours. This expression indicates half-times for the respective retention coefficients of ~20 min, 0.8 hr and 47 hours. This equation is not, of course, a unique description of the data; however, it takes into account the emphasis in the first study (Morrow et al., 1980) on the earliest postexposure times for estimating the most rapid retention coefficient.

D. Intravenous Studies

The nine intravenous studies provide comparable tabular and graphic summaries, viz., Table 3 and Figure 5, respectively, to those prepared for the inhalation studies.

The main similarities in findings from the two types of studies include: (a) comparable cumulative excretion pattern (24-336 hrs): 56-89 percent for the average I.V. and 66-92 percent for the average inhalation study; and (b) similar renal retention of uranium: 16 to 6.5 percent of the injected dose for 24 to 336 hour values in the intravenous studies and 16 to 5.5 percent of the absorbed dose over the same period of the inhalation studies.

The major distinctions found between the two routes of administration were: (a) the much greater fecal elimination after inhalation, viz., 16 percent vs 2 percent by 336 hours and (b) the slightly higher mean body burden 24 to 336 hours after intravenous administration viz., 44 percent to 11 percent, compared to 34 to 8

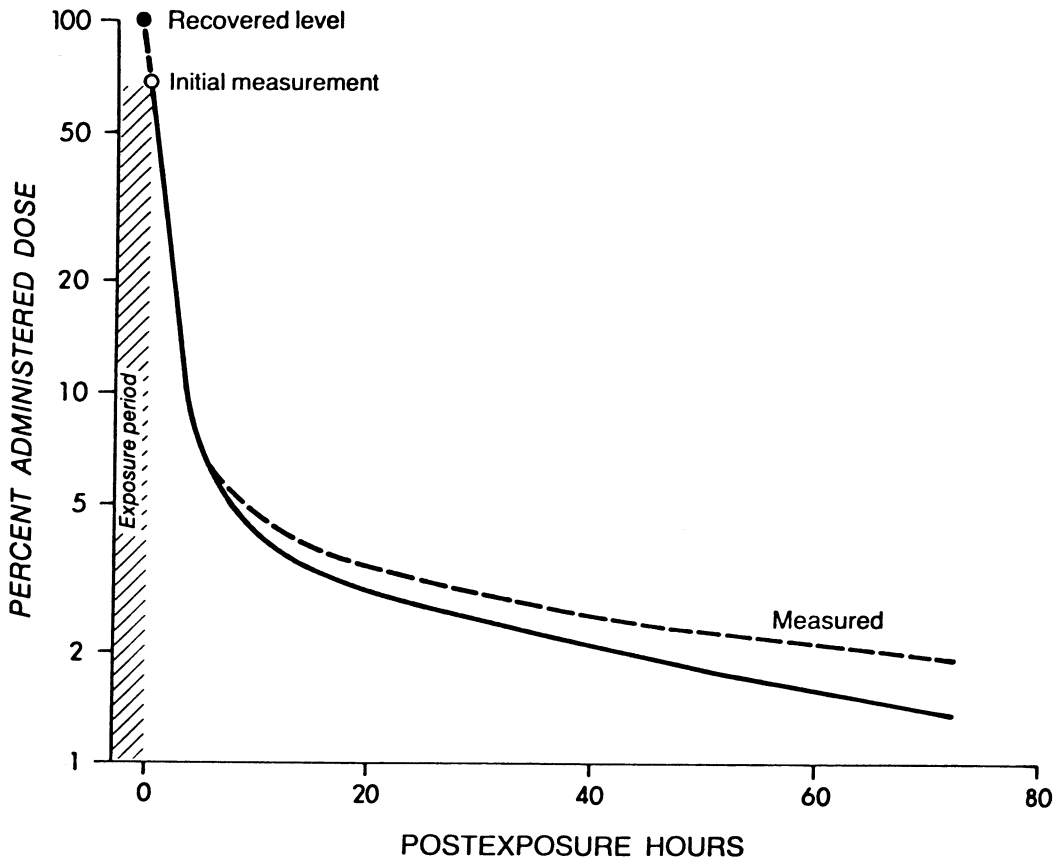


Figure 4. UO_2F_2 Retention in the Lungs.
The curve commencing with "initial measurement" and marked "measured" depicts the lung retention information acquired. After an adjustment is made for UO_2F_2 clearance during the exposure, which established the "recovered level", and for the extrapulmonary U^{+6} included in the thoracic counts, the "corrected" retention curve was constructed.

Table 3: Uranium disposition following intravenous injection (9 studies)

	Percent of Injected Dose				
Time after Injection (Hours)	24	48	72	144	336
Mean Cumulative Excretion	56	69	78	83	89
(Standard Error)	(4.4)	(4.3)	(8.6)	-	-
% in urine	100	100	99	95	99
% in feces	0	0	1	5	2
Mean Body Burden	44	31	22	17	11
Kidneys	16	22	14	9.5	6.5
Skeleton*	4	5	6	6	4.5
Lungs	0.5	0.1	-	-	-
Other	23.5	3.9	2	1.5	-

* wt assumed equal to 0.15 body wt.

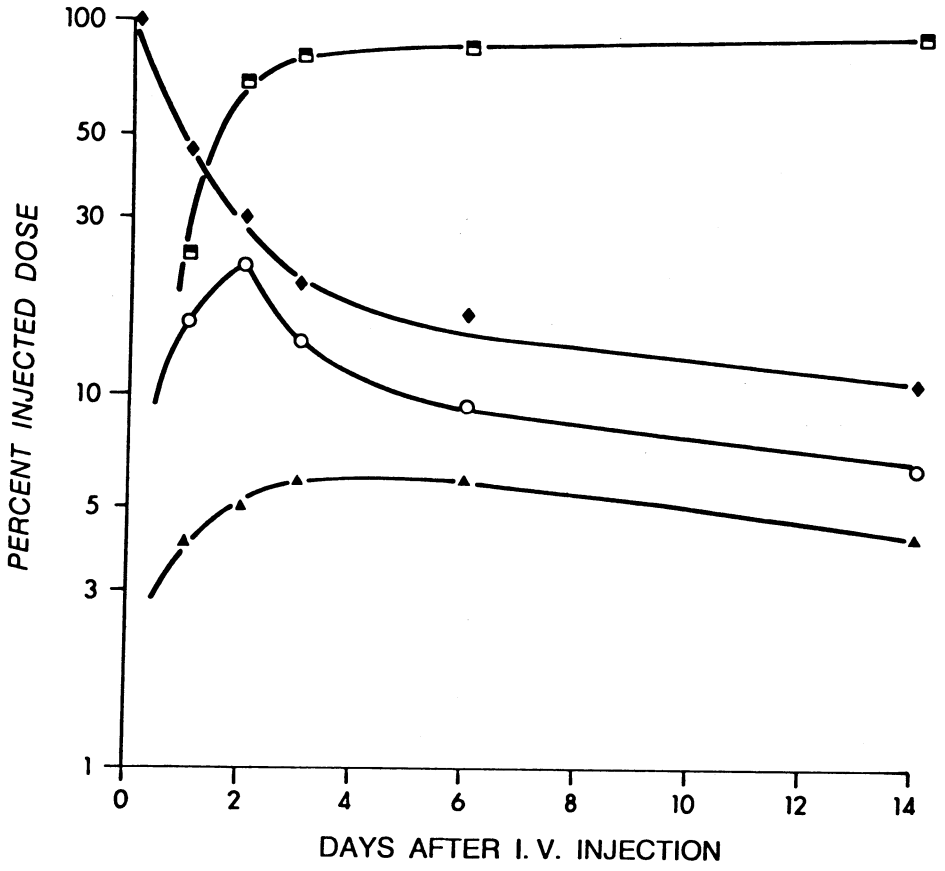


Figure 5. Intravenous Studies: Distribution and Excretion. The cumulative total excretion (■) and urinary excretion of U^{+6} (□) are depicted as being virtually identical. The symbols for kidney content (O) and estimated bone levels (▲) are the same as in Figure 3. The body burden curve determined from the cumulative excretion data is denoted by (◆).

percent after the inhalation. The urinary/fecal excretion ratio is clearly the more striking distinction; the intravenous studies suggest that approximately 3/4 of the fecal content following the inhalation exposures (~13 percent of the administered dose) was due to translocation of unabsorbed uranium from the respiratory tract, whereas, the remaining 1/4 (~5 percent) was due to the redistribution of absorbed uranium: this was the fecal level which occurred in the intravenous subjects probably derived from biliary excretion.

In addition to the similarities seen between the inhalation and intravenous studies in dogs, there is general agreement with the 24 hour and 5 day human data wherein 57-83 percent and 70-90 percent urinary elimination respectively, occurred in those subjects receiving 0.16 mg U kg^{-1} or less intravenously. Additionally, the human tissue data gave 16.6, 7.2 and 0.7 percent renal retention of uranium at 2.5, 18 and 74 days post administration, respectively (Hursh and Spoor, 1973). There is excellent agreement over the early time period with the renal data in Table 3.

In one animal (Table 1: Studies V1/V2) a dose of 0.01 mg U kg^{-1} was injected intravenously five times at ~6 day intervals. A final dose of 0.45 mg kg^{-1} was given on day 31. Each of these injections caused a marked, but transient glucosuria which returned (4 out of 5 times) to control levels before the next injection was given (Figure 6). A less cyclic proteinuria of questionable significance was noted. Also, each dose of uranium led to slightly less U excretion (incremental) in the urine over the 6 days following. The final, large intravenous dose showed that the kidney was further injured by uranium (glucose and GFR) but the proteinuria was again somewhat equivocal. The most significant result of this study was clearly the evidence of renal tubular dysfunction (glucosuria) in response to a 10 μg kg^{-1} absorbed (injected) dose. From the Figure 5 data, one can deduce that in a 8 kg dog having 40 g kidneys, a transient peak renal concentration $\leq$1 μg U g^{-1} might result from such an absorbed dose.

E. Urinary Biochemistry

An overall evaluation of each urinary indicator of renal injury used in the dog studies was made and is summarized as follows:

(a) Protein: Elevated protein levels in the urine (mg day^{-1} or μg ml^{-1}) were a good index of renal injury, often increasing as early as 1 day but usually 2 to 3 days after UO_2F_2 administration. With the highest level inhalation exposures or intravenous injections, transient proteinuria was often seen within a few hours of the time of UO_2F_2 administration. The overall course of proteinuria following UO_2F_2 exposure was dose-dependent: the larger the dose the higher and more persistent the proteinuria. Major limitations found with protein determinations in the dog were two-fold: the relatively high and variable levels of protein in the control urines,

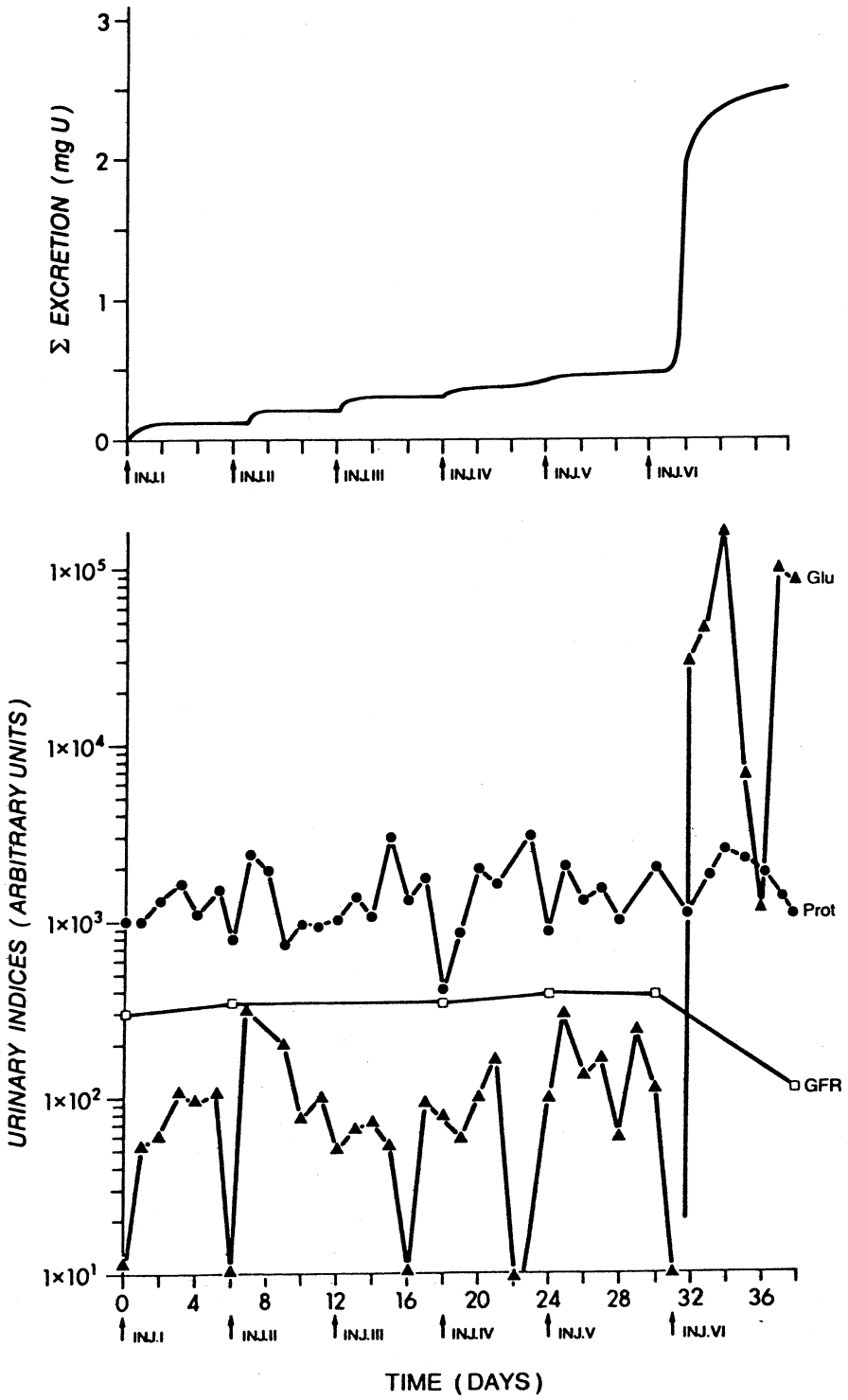


Figure 6. Responses to Multiple Intravenous Doses of UO_2F_2.
Top: The cumulative urinary recoveries for U^{+6} after five identical intravenous injections show some degradation with time. The terminating large single dose resulted in a diminished urinary excretion rate for U^{+6} compared to single-injection dogs in the same dose range.
Bottom: These data from the same animal depicted above show that the more sensitive biochemical indicators respond to 0.01 mg kg^{-1} doses.

(e.g., 1903 μg albumin ml^{-1} urine, Standard Deviation (S.D.) 953 μg; N = 10); and the comparatively low ratios between exposed and control animal urinary protein levels: these rarely exceeded a factor of 10, even at the highest dose levels.

(b) Plasma Urea Nitrogen (PUN): This plasma indicator was somewhat less sensitive than protein in the urine giving exposed/control ratios of less than 5 for the most part. It was not elevated in some low uranium dose animals which manifest other biochemical changes to a significant degree. When seen, PUN elevation was often more persistent than proteinuria. Control levels were 88 μg urea N ml^{-1} plasma, but not nearly so variable (S.D. ~20 percent).

(c) Alpha Amino Nitrogen: This renal injury index expressed in μM day^{-1} was found to track urinary protein elimination quite closely. Its principal advantage over protein was that the exposed/control ratios were somewhat higher. The control mean was 12,400 μM glycine equivalents day^{-1}; S.D. 6,200 μM.

(d) Glucose: Of all the urinary changes assessed, the increase in glucose elimination was the most impressive. Control levels were consistently very low (control mean 53 μM day^{-1}; S.D. 81 μM), and the exposed/control ratios usually exceeded 10^4 at the higher doses (>0.5 mg U kg^{-1}). The appearance of glucosuria was always as prompt after U exposure as proteinuria or any other indicator change. The persistence of significant glucosuria usually exceeded that for protein or α-amino acid N elimination, although the peak levels for all three might occur about the same time, i.e., 2-4 days postexposure.

(e) Inulin Clearance (glomerular filtration rate): With methoxy-C-14-inulin, this was not a particularly tedious method although it did require anesthetized, catheterized dogs. Control values were consistent, viz., 3.9 ml min^{-1} kg^{-1}, S.D. 0.78, so that reductions to 50 percent of control values were highly significant; often exposed dogs showed GFRs which were 10 percent or less of normal. The subsequent recovery of the GFR to normal levels was consistently more prolonged than for a return of protein and most other indicators. The recovery of the GFR commonly required 2 weeks; in some cases it did not recover completely but remained at 70 to 80 percent of control values, implying that some persistent glomerular deficit occurred.

(f) Phosphate: This indicator of uranium-induced renal injury was not particularly useful in the dog since excretory levels (μg ml^{-1} or mg day^{-1}) were highly variable among controls (e.g., 100-500 mg day^{-1}) and exposed animals, with the latter rarely increasing more than 2-4 times the average control level. Large numbers of samples would be required to use this index with suitable reliability.

(g) N-acetyl-β-glucosaminidase (NAG): This urinary indicator of renal injury has been advocated for human and rodent use, but prior to our studies had not been examined in the context of uranium toxicity in any specie. Its use seems promising although the daily units excreted are relatively high and variable (mean 5051) among control dogs. However, the exposed animals exceeded these units/day values often by factors of 10^1 to 10^3 and also the NAG levels tended to remain elevated for longer times than many other urinary indicators.

(h) Citrate: This index was examined more thoroughly in the rodent experiments than in the dog. In 12 canine determinations, the control values averaged 174 μM day^{-1} and after UO_2F_2 exposure or I.V. injection, the citrate levels fell below 100, often to near zero, after a transient rise (2-3 fold). The higher the dose, the sooner and more persistent the fall in citrate elimination. At the lower doses of UO_2F_2, the maximum depression in citrate level occurred 5-8 days post administration. We found no consistent evidence of increased citrate excretion during or after tubular regeneration in contrast to Haven (1947).

(i) Creatinine: Plasma creatinine (mean 9.6 μg ml^{-1} plasma) was followed in our initial dog experiments but with the institution of plasma inulin clearance, was discontinued. In the earlier Rochester studies reported by Berke et al. (1953), amino acid nitrogen and creatinine excretion in the urine were set as a ratio to reduce their variabilities since both include a urinary flow dependence; otherwise, the variabilities found in animal data were very large and difficult to interpret. Our limited experience concurs with that appraisal although we did not attempt to employ the ratio.

If the various plasma and urinary indices are compared in relation to absorbed dose, there are trends which are more or less proportionate, but there are many exceptions as well. One of the confounding features relates to the variable time to a maximal response among different indices, doses and subjects. If, on the other hand, indices from the absorbed or injected dose range, 0.22 to 0.78 mg U kg^{-1}, are pooled on the basis of maxima occurring within the first 3 to 5 days post administration, the mean maximal responses in exposed dogs can be compared in terms of their mean control values using a student's t test (Table 4). This analysis of selected indices indicates that in spite of considerable variability, and often unclear dose-response relationships, some indices, e.g. α-NH_2 nitrogen, manifest sufficient change as to be highly significant from controls, while others e.g. PUN and glucose, are both less variable and more responsive.

Summarizing the results of the canine urinary biochemistries is difficult in view of the various dose and time protocols. The problem is illustrated by the time course of two typical studies

Table 4: Exposed vs control mean indices in dogs

	Control		Exposed		t test
Urine volume ($ml\ day^{-1}$)	mean 286	S.D. 76	mean 558	S.D. 394	<0.02
Protein ($mg\ BSA\ day^{-1}$)	1 165	716	3 430	1 457	<0.001
Glucose ($\mu m\ day^{-1}$)	53	81	8 800	6 830	<0.001
α-NH_2 (μM glycine eq. day^{-1})	12 400	6 200	39 150	42 000	<0.001
Citrate ($\mu M\ day^{-1}$)	174	250	99	209	<0.10
NAG (units day^{-1})	5 900	4 100	177 000	359 000	<0.10
PUN* (ug urea N ml plasma)	89	20	620	382	<0.001

* based on maxima in 5-7 day interval

depicted in Figure 7. One is based on the most interesting of the renal injury indicators from a dog (study I-15 of Table 1) which received by inhalation a recovered dose of 0.78 μg U kg^{-1} (Figure 7, Top). Most of the indices rose to significant (abnormal) levels by day 2, except plasma creatinine and urinary NAG, and, of course, the GFR which decreased significantly. All indices manifest abnormal levels by day 3. The 0.78 μg U kg^{-1} dose was definitely toxic to the kidneys and most of the indicators remained significantly abnormal for 5 to 6 days. By 14 days, all indicator values had returned to normal with the exception of urinary NAG, urinary glucose (although it has dropped 100 fold from peak levels) and the GFR which was 70 percent of control level and probably depressed significantly ($p < 0.10$).

In Figure 7 (Bottom) the biochemical indicators from a second inhalation animal (HF 2) are depicted. This dog received a combined UO_2F_2 and HF exposure which resulted in a total recovered dose of 0.55 mg U kg^{-1} (Table 1). Here several differences from study I-15 are noteworthy: the urinary NAG level was more variable and returned to the normal range in 8 days; the GFR never recovered fully in the 16 day period (50 percent of control value); urinary glucose increased even more in this animal and it remained significantly elevated for at least 16 days post-exposure; and proteinuria was marked and sustained over the entire period. These quantitative distinctions occurred at a lower UO_2F_2 dose suggesting that the nephrotoxicity of UO_2F_2 with HF may be greater than with UO_2F_2 alone. Unfortunately, too few dogs were studied to establish this observation as a firm conclusion. The rodent studies also examined the combined exposures for evidence of synergism (See Section VI.2.F.).

F. Multiple Dose Studies: Tolerance to Uranium-induced Renal Injury

In Figure 8 (Top) excretion data are summarized from an intravenous study (V5) wherein a dog received a 0.44 mg U kg^{-1} dose and then after 14 days was reinjected at the same dose level (See study VI-6, Table 1). The Figure 8 (Top) data show the cumulative uranium excretion characteristics following both injections. In studies V5/V6 the total injected dose was 3.58 mg U X 2 and the initial 24 hour elimination was 1.47 mg U (~42 percent of the injected dose), whereas, only 1.19 mg U (33 percent of the injected dose) appeared in the urinary excretion over the same time period after the second injection. No change in the fecal excretion of uranium was noted.

Figure 8 (Bottom) summarizes some of the renal injury indices in this same dog. It is readily seen that urinary protein and glucose levels increased significantly after both injections and returned to normal in 14 days. The second injection occurred after a dose and time interval which should have produced "tolerance". Whether the slightly reduced peak levels of glucosuria and proteinuria are significant or not is dubious. The GFR, interestingly, provides a contrasting picture despite the less frequent determinations. At 7

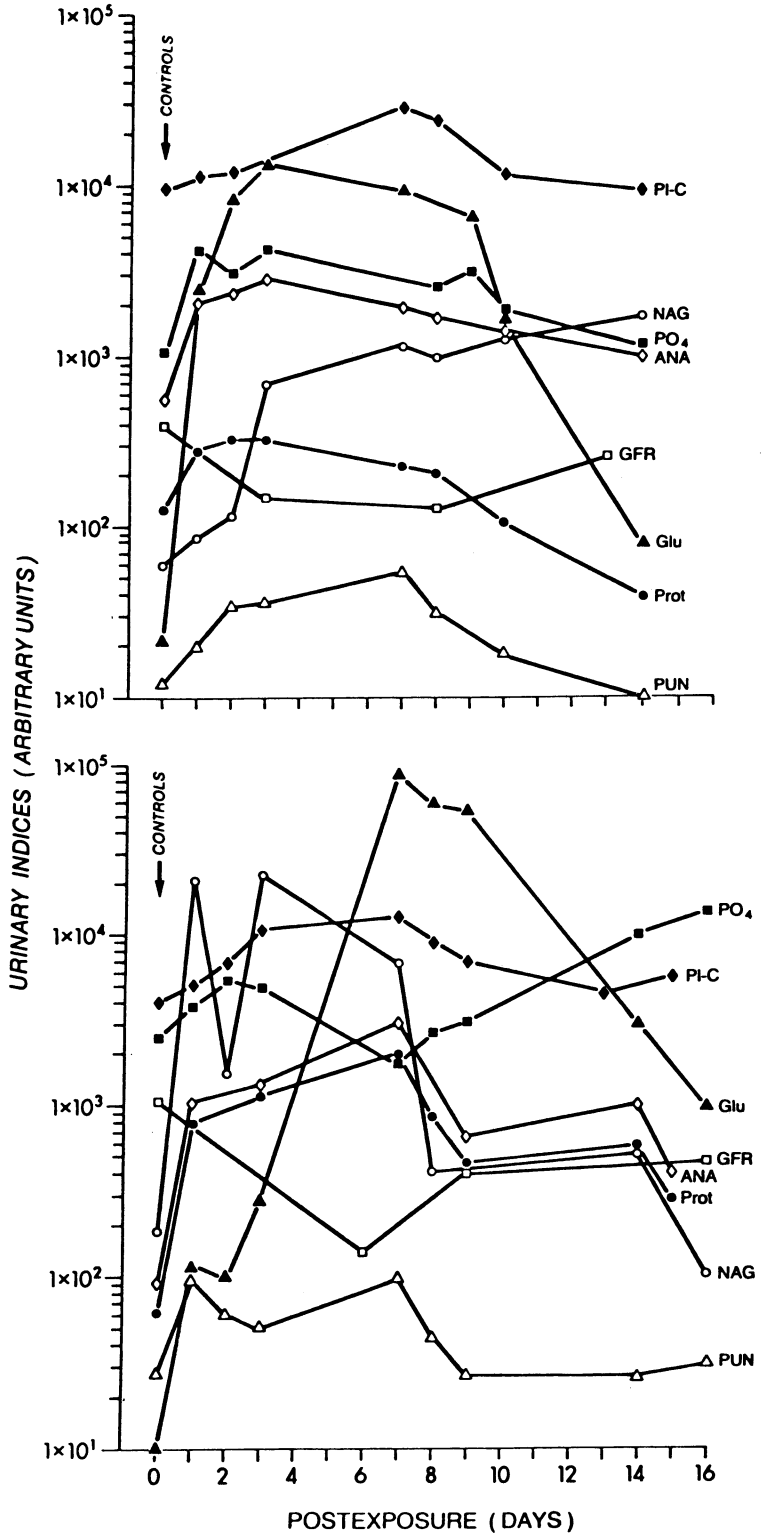


Figure 7. Biochemical Indices: Representative Inhalation Studies. The upper figure is based on plasma and urinary data from inhalation study I-15. The lower figure shows similar data from a dog which received a combined HF-UO_2F_2 exposure resulting in about 70 percent as much UO_2F_2 deposition as in the I-15 dog.

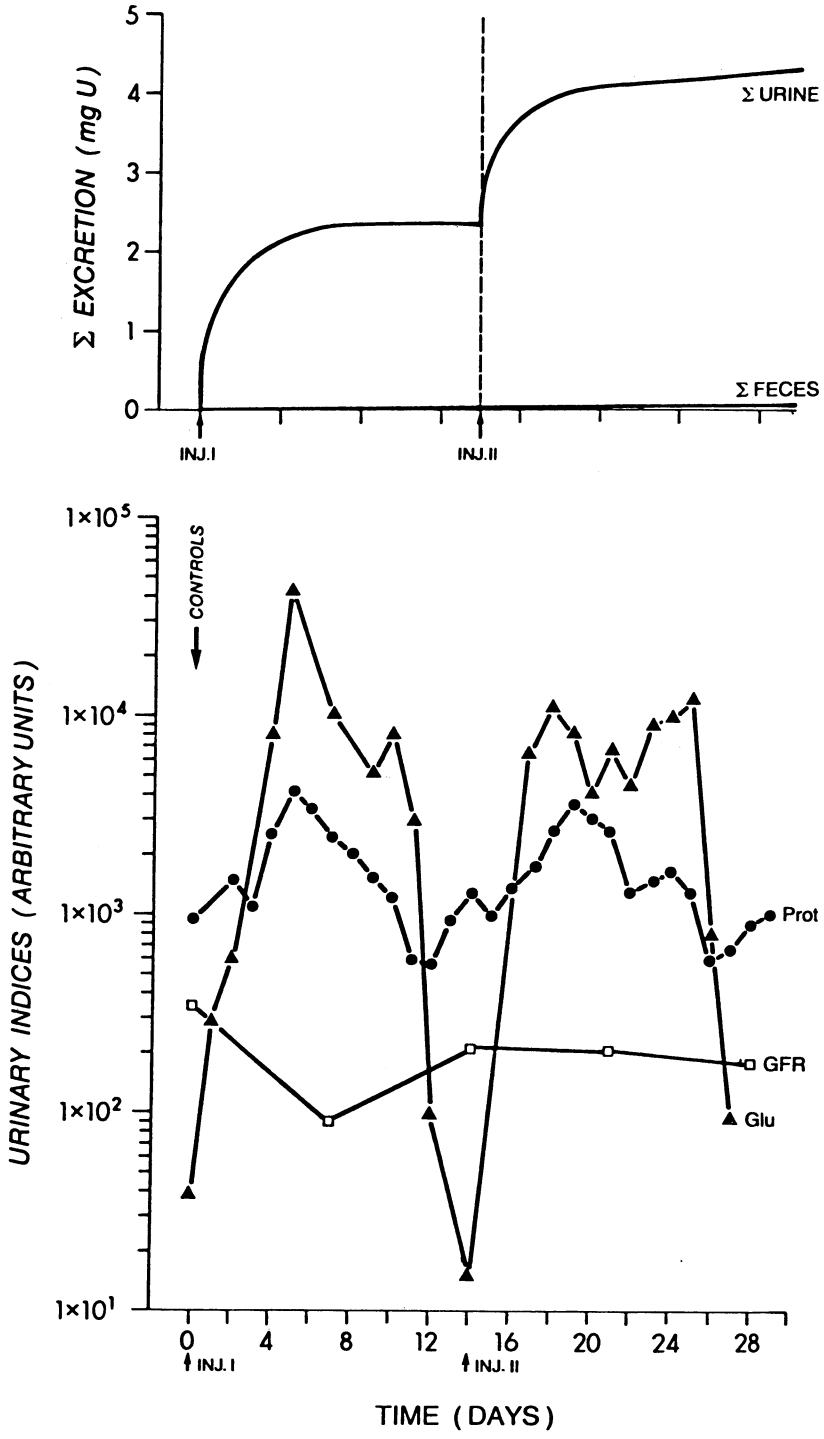


Figure 8. Responses to Two Intravenous 0.44 mg/kg^{-1} Doses.
Top: The cumulative urinary recovery of U^{+6} following two identical intravenous doses is shown. The recovery after the second injection was only 81 percent of the first over comparable time periods.
Bottom: These biochemical data were obtained in the same dog following two i.v. doses of aqueous UO_2F_2 separated by 14 days during which all indicators of renal tubular function returned to normal. The zero time values are average control values.

or 8 days post administration, the maximal reduction in GFR is normally encountered. After the first injection the GFR fell 75 percent at 7 days, and did not fully return to normal in 14 days remaining 39 percent below control values. After the second injection, the GFR manifested no further change (posssibly a slight reduction) at the +7 days post injection as though further glomerular injury was not effected. Renal histology in this dog revealed evidence of extensive tubular damage with extensions to the capsule in many areas: necrotic debris was absent and tubular regeneration was advanced, generally.

The other multiple-injection study (V1/V2) which was discussed in the context of renal injury threshold (Figure 6) can also be assessed in terms of naive versus previously-exposed subjects. This same subject showed an increased urinary output which seemed to be associated with each injection (Figure 9). The association seen in biochemical parameters, uranium excretion, urinary volumes and dose, seemed clear initially, but became less evident with successive doses. The general picture which emerges is also consistent with the data in Figure 7, in that both subjects' responses are variable temporally and quantitatively depending upon the assessment criteria.

G. Special Studies

(1) A pilot study to examine the possible induction of a uranium-protein complex in the kidneys of multiply-dosed dogs was undertaken. In this case, one dog (9.3 Kg, female) was given five weekly doses of uranyl fluoride synthesized from natural uranium: each dose was 0.1 mg U kg^{-1} administered intravenously. A comparison dog (8.7 kg) was given five 1 mg Cd kg^{-1} doses of cadmium sulfate intravenously using a similar protocol. This cadmium dose regimen would be expected to induce renal metallothionine (Onosake and Cherian, 1981). Six days following the fifth weekly dose, both dogs were given 0.5 mg U kg^{-1} of U-235 (93 percent) uranyl fluoride intravenously and sacrificed at identical post-injection times (+6 days) in order to determine if the U-235 retention by the kidneys was comparable. Halves of each dog kidney were combined as a specimen and frozen in liquid nitrogen. They were later homogenized in TRIS buffer, ultracentrifuged and fractionated on a 80 cm G-75 Sephadex column at 72 ml hr^{-1}. In Figure 10, the cumulative urinary uranium excretion is plotted for the two dogs and their terminal renal uranium concentrations are depicted. Greater urinary uranium excretion and lower renal uranium concentrations are manifest by the uranium-pretreated dog. Conversely, the cadmium-pretreated dog's greater renal retention of uranium is striking, but the cytosol from this dog's kidney was not appreciably different from the uranium-pretreated dog, exhibiting neither a concentration of uranium nor any evidence of metallothionine association with uranium, nor a different pattern of protein induction (Figure 11).

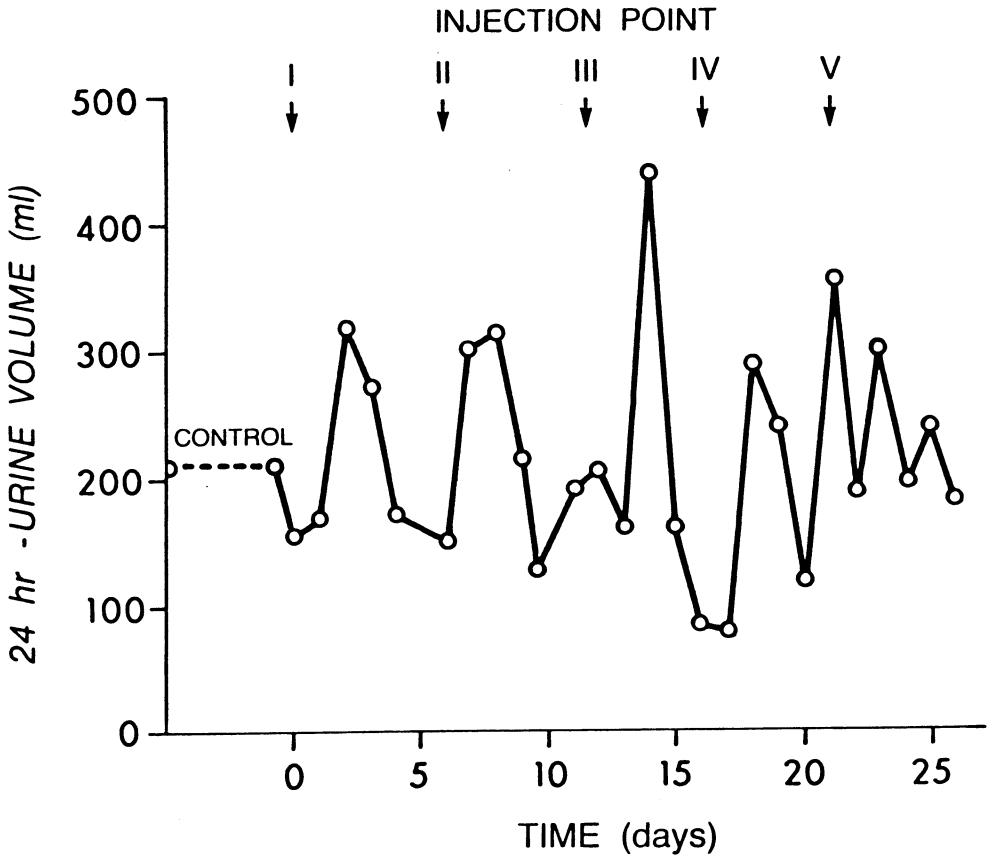


Figure 9. Urinary volumes after Intravenous UO_2F_2.
After multiple doses of 10μg U kg^{-1}, each subsequent day's urine volume was measured. As with other urinary parameters, each injection resulted in an increased urinary output, but the effect became less discrete after several UO_2F_2 administrations.

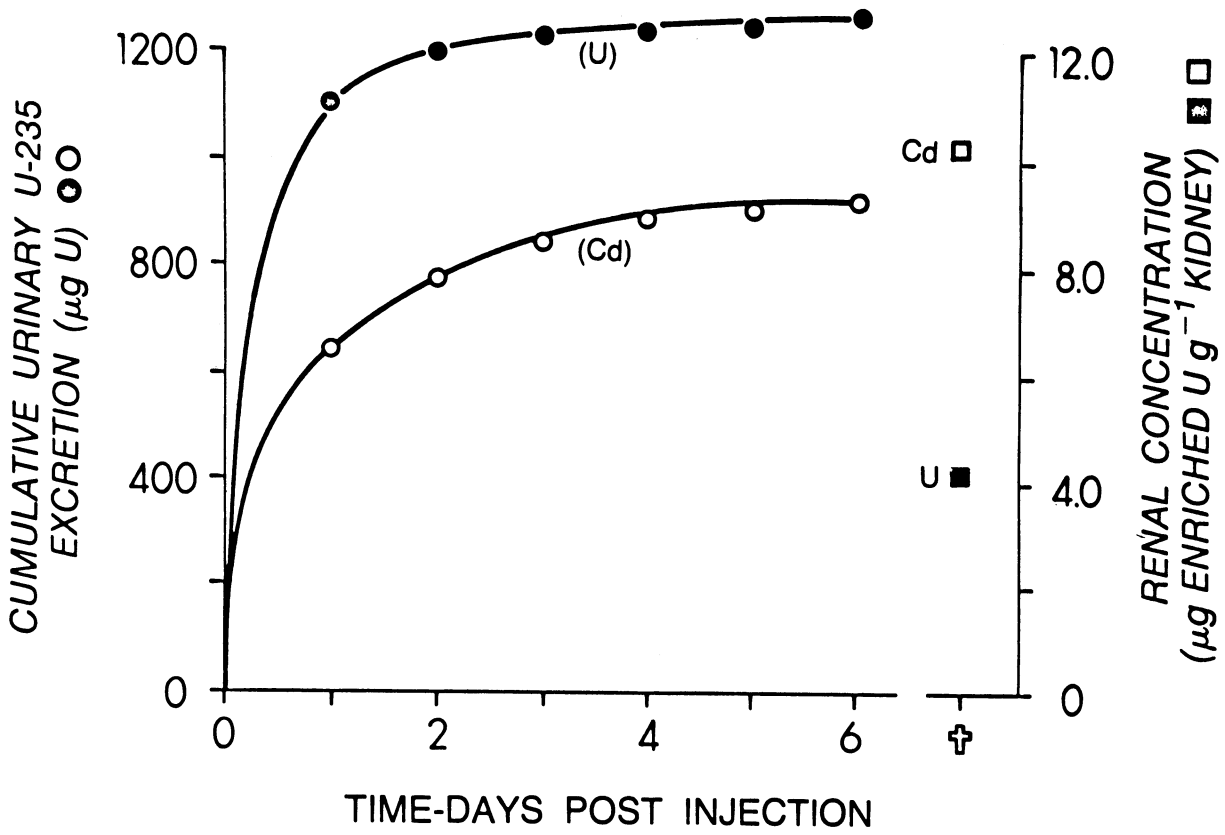


Figure 10. Pilot Study of Possible Renal Protein Induction:Uranium Excretion. Using the protocol described in the text, the cadmium (Cd) and natural uranium (U) dogs given the same dose of $^{235}UO_2F_2$ at time zero showed the cumulative urinary uranium-235 output as depicted by the curves (left axis). The terminal (✞) kidney burdens of the same dogs are also depicted (right axis).

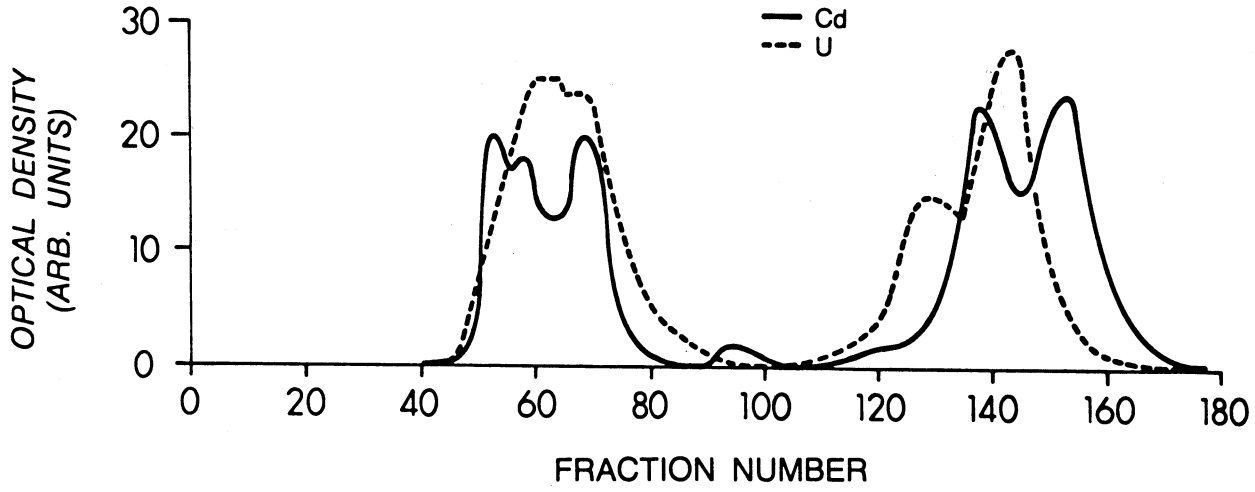


Figure 11. Pilot Study of Possible Renal Protein Induction: Protein Pattern. The kidneys of the cadmium and natural uranium treated dogs were examined chromatographically and no major distinctions were noted. Although metallothionine induction was probable in the Cd dog, it appeared to have no clear association with U-235, notwithstanding increased U-235 retention by the kidneys of the Cd treated dog.

H. Histopathology

The renal injury observed in the dogs from the inhalation and intravenous studies was comparable: all dosage protocols used were injurious to the kidneys. At between 1 and 3 days, their kidneys showed widely scattered necrosis of segments of the deep convoluted tubules and the straight portions of the corticomedullary junctions extending toward the mid-cortex and involving most tubuli renales recti. The tubules contained hyaline material and proteinaceous casts and some collecting tubules contained calcified debris. The tubular epithelial cells were generally pale, often without nuclei and denuded. The glomerular capillaries were generally congested. The extent and severity of these changes were related to absorbed dose only at similar times after exposure.

Up to three days, evidence of tubular regeneration was rarely seen, but after 6 days it was seen in all dogs, especially those at the lower recovered or injected doses (<0.44 mg kg^{-1}), typified by increased mitotic figures and development of a flattened tubular epithelium. At the lowest single doses studied, 0.11 mg kg^{-1} and 0.12 mg kg^{-1} (studies I-1 and V-3), kidneys from both dogs revealed uranium-induced injury, but both were undergoing extensive regeneration at sacrifice 6 days after exposure while having renal concentrations of 1.5 and 1.6 μg U g^{-1} kidney, respectively, at that time. This was equivalent to an average renal burden of 9.2 percent of the dose (Tables 2 and 3).

In the dogs receiving the higher absorbed and injected UO_2F_2 doses, residual manifestations of tubular dilation and atrophy were present in dogs sacrificed 14 and 19 days after exposure. Collectively, the higher dose animals provide evidence for persistent tubular damage in the presence of largely regenerated renal tissue which itself differed from normal kidney by the emergence of flattened, basophilic, tubular epithelium.

2. Rodent Studies (Leach and Gelein)

A. Exposures

In our earlier report, NUREG/CR-1045 (Morrow et al., 1980), we examined the toxicologic properties of one of the hydrolysis products of UF_6, namely particulate UO_2F_2. Several findings from this work warrant review since they form the basis on which the toxic action of UO_2F_2 can be compared with or without the presence of HF, the other component of hydrolyzed UF_6. For example, summarized in Table 5 is selected information obtained from experiments with rats given UO_2F_2 by various routes of administration. In the inhalation studies (Experiments No. 3 through 6), rats showed high fecal excretion of U, that is, 59 to 72 percent of the inhaled dose, upon nose-only exposures to UO_2F_2 aerosols ranging in particle size from about 1 to 2 μm MMAD (σ_g ~1.9).

EXPERIMENT NO.	ROUTE OF ADMINISTRATION	DURATION OF EXPOSURE, hr	MEAN AEROSOL CONC., mg U m^{-3}	PARTICLE SIZE DATA		MEAN INHALED DOSE, mg U kg^{-1}	MEAN DOSE GIVEN, mg U kg^{-1}
				MMAD, µm	σ_g		
1	INHALATION	1	CONTROL EXPER.*				
2	INHALATION	2	CONTROL EXPER.*				
3	INHALATION	2	204±16	1.72	1.79	10.4	
4	INHALATION	1	234±15	1.40	1.94	6.1	
5	INHALATION	1	174±13	1.18	1.87	3.6	
6	INHALATION	1	63±05	1.00	1.85	1.4	
7	INTRAVENOUS						5.7
8	INTRAVENOUS						0.6
9	INTRATRACHEAL						6.3
10	GAVAGE						8.1

EXPERIMENT NO.	NO. OF RATS	DAYS AFTER TREATMENT	MEAN ABSORBED DOSE, mg U kg^{-1}	MEAN PERCENT OF ABSORBED DOSE OF U				FECAL U AS A MEAN % OF INHALED OR GIVEN DOSE
				URINE	BONE	KIDNEYS	LUNGS	
1	6	30	CONTROL EXPER.*					
2	6	30	CONTROL EXPER.*					
3	14	6	3.7	43	43	11	3	60
4	6	14	2.4	68	23	5	3	59
5	8	7	1.1	48	45	5	0	69
6	6	7	0.4	73	27	0	0	72
7	24	10	5.3	38	45	16	0	5
8	4	7	0.6	35	52	13	0	6
9	12	7	4.6	36	38	23	3	29
10	2	7	0.1					99

*Control experiments to determine "nose-only" exposure stress and normal toxicologic indexes.

Table 5. A summary of selected information obtained from experiments with rats given UO_2F_2 by various route of administration (see NUREG/CR-1045).

From the large amounts of U found in the feces we deduced that the rat, an obligatory nose breather, trapped UO_2F_2 aerosols in the nasopharyngeal region quite efficiently and these are promptly swallowed and passed through the GIT. Since U^{+6} compounds are poorly absorbed from this tract, the UO_2F_2 is almost quantitatively excreted in the feces (see Table 5, Experiment No. 10); in the case of these earlier experiments this accounted for more than 50 percent of the inhaled dose. We believe that the remaining 28 to 41 percent of the inhaled dose of U was absorbed through the respiratory tract and promptly translocated to bone and kidney, 15 to 27 percent of the inhaled dose eventually being excreted as urinary U.

In the new work reported here five inhalation studies were conducted to establish the excretion, distribution and organ retention patterns of U in rats upon exposure to hydrolyzed UF_6 (UO_2F_2 aerosol + HF gas). Exposure levels were chosen high enough (mean UO_2F_2 aerosol concentrations ranging from 284 to 816 mg U m^{-3} for 1 to 1.5 hours duration) to follow U in urine, feces and organs of the body for at least several days, by gamma counting, and to produce kidney injury demonstrable by biochemical assays. These studies are identified in Table 6 as Experiments No. 11 through 15, respectively.

Both natural and enriched (93.15 percent ^{235}U) UF_6 were used in these studies depending on the type of information desired. For example, the inhalation study designated as Experiment No. 15 (see Table 6) was conducted to roughly mimic Experiment No. 11, except that natural UF_6 was used instead of enriched. At these lower radiation levels (1/70 alpha activity), the effects of U on the kidneys were traced by various renal injury indicators.

The last three experiments (Nos. 16, 17 and 18) listed in Table 6 were multiple-dose intravenous studies designed primarily to demonstrate the behavior of the rat kidney which was previously exposed to U^{+6} in the form of UO_2F_2.

In the graphs that follow, each plotted point represents a mean value obtained from the number of animals noted; error bars, where shown, are standard deviations.

B. Uranium Distribution and Excretion

The results of Inhalation Experiments No. 11-14 are summarized in Tables 7, 8, 9 and 10. These data include calculated values for inhaled dose, absorbed dose and percent of absorbed dose of U found in the urine, carcass (bone), lungs and kidneys of rats exposed once for 1 or 1-1/2 hours to the hydrolysis products of UF_6.

It is interesting to see the similarity in the distribution patterns of U (expressed as a mean percent of the absorbed dose) in animals from Experiments No. 11, 12 and 13 even though the duration of exposure, mean air concentration of U or aerodynamic particle size

Table 6. A list of selected rat experiments conducted with hydrolysis products of enriched (FE) or natural (nat) UF_6

EXPERIMENT NO.	NO. OF RATS	ROUTE OF ADMINISTRATION	DURATION OF EXPOSURE, hr	MEAN HF CONC., mg HF m^{-3}*	MEAN AEROSOL CONC., mg U m^{-3}	PARTICLE SIZE DATA MMAD, μm	σ_g
11	11	INHALATION	1.5	120	357±89 (FE)	1.59	1.92
12	7	INHALATION	1	238	710±82 (FE)	0.88	1.62
13	6	INHALATION	1	243	726±289(FE)	1.07	1.73
14	37	INHALATION	1	273	816±98 (FE)	1.80	1.61
15	8	INHALATION	1	95	284±39 (nat)	0.88	1.91
16	3	INTRAVENOUS	MULT. DOSE[1]		(FE)		
17	6	INTRAVENOUS	MULT. DOSE[2]		(FE)		
18	6	INTRAVENOUS	MULT. DOSE[3]		(FE)		

1. Control experiment where saline solution (0.2 ml 100 g^{-1}) was given at 6 day intervals for 6 times, then 5 mg U kg^{-1} was given as UO_2F_2.

2. 0.1 mg U kg^{-1} was given as UO_2F_2 at 6 day intervals for 6 times, then 5 mg U kg^{-1} was given as UO_2F_2.

3. 1 mg U kg^{-1} was given as UO_2F_2 at 14 day intervals for 3 times, then 5 mg U kg^{-1} was given as UO_2F_2.

* HF concentrations based on stoichiometric coefficients of the following reaction: $UF_6 + 2\ H_2O \rightarrow UO_2F_2 + 4\ HF$. The actual exposure concentrations were probably lower due to the reactivity of HF and problems associated with its measurement in the presence of UO_2F_2 aerosol.

ANIMAL NUMBER	INHALED DOSE		ABSORBED DOSE		MEAN PERCENT OF ABSORBED DOSE OF U			
	mg U	mg U kg^{-1}	mg U	mg U kg^{-1}	URINE	BONE	KIDNEYS	LUNGS
01	1.93	9.01	0.61	2.85	52	39	5	4
02*	1.58	7.80	0.64	3.18	40	51	7	2
03	1.76	7.65	0.50	2.18	58	35	3	4
04	1.15	5.01	0.47	2.04	56	33	5	6
05	2.15	9.36	0.66	2.87	54	39	4	3
06	1.38	6.29	0.49	2.24	58	32	6	4
07**	0.84	3.93	0.23	1.10		54	6	40
08	1.44	7.20	0.63	3.16	50	39	5	6
09	1.53	6.82	0.50	2.24	52	38	6	4
10	1.84	8.53	0.53	2.44	54	35	6	5
11	1.85	8.40	0.49	2.23	46	40	7	7
MEAN	1.67	7.59	0.54	2.47	53	37	5	5
SDEV	0.32	1.41	0.07	0.39	4	3	1	1
N=9								

*Animal died on eighth postexposure day. ** Animal died during 1.5 hr exposure. Except for these two animals all other of the nine were sacrificed on the 14th postexposure day; their data comprise the mean and sdev values.

Table 7. Experiment No. 11. The inhaled dose of U, absorbed dose and distribution of absorbed dose in LE rats exposed for 90 min. to the hydrolysis products of UF_6 at an air concentration of 357±89 mg U m^{-3}, with a particle size of 1.59 μm MMAD (σ_g = 1.92).

ANIMAL NUMBER	POSTEXPOSURE DISPOSITION	INHALED DOSE		ABSORBED DOSE		PERCENT OF ABSORBED DOSE OF U			
		mg U	mg U kg^{-1}	mg U	mg U kg^{-1}	URINE	BONE	KIDNEYS	LUNGS
12	A	1.27	5.54	0.62	2.69	53	40	3	4
13	B	1.27	5.74	0.79	3.54	23	59	14	4
14	C	1.28	5.66	0.66	2.91	41	49	5	5
15	D	1.50	6.25	0.81	3.36	41	47	8	4
16	A	1.82	8.27	0.93	4.22	49	36	7	8
17	E	1.20	5.24	0.62	2.68	52	39	5	4
18	A	1.56	6.91	0.79	3.50	47	36	8	9

A. Animals sacrificed on postexposure day 21.
B. Animal died on postexposure day 2.
C. Animal died on postexposure day 19.
D. Animal died on postexposure day 8.
E. Animal died on postexposure day 14.

Table 8. Experiment No. 12. The inhaled dose of U, absorbed dose and distribution of absorbed dose in LE rats exposed for one hr to the hydrolysis products of UF_6 at an air concentration of 710±82 mg U m^{-3}, with a particle size of 0.88 μm MMAD (σ_g = 1.62).

ANIMAL NUMBER	INHALED DOSE		ABSORBED DOSE		PERCENT OF ABSORBED DOSE OF U			
	mg U	mg U kg^{-1}	mg U	mg U kg^{-1}	URINE	BONE	KIDNEYS	LUNGS
19	1.10	4.63	0.34	1.43	44	41	6	7
20	1.79	7.30	0.72	2.96	58	35	3	4
21*	1.82	7.37	1.23	4.98	39	46	11	4
22	1.85	7.77	0.78	3.27	58	37	3	2
23	1.54	7.45	0.73	3.53	63	31	2	4
24	2.13	10.06	0.93	4.41	57	38	3	2
MEAN	1.68	7.44	0.70	3.12	56	36	3	4
SDEV	0.39	1.93	0.22	1.09	7	4	2	2
n=5								

* This animal died on the seventh postexposure day, all others were sacrificed on the fifteenth postexposure day.

Table 9. Experiment No. 13. The inhaled dose of U, absorbed dose and distribution of absorbed dose in Le rats exposed for one hr to the hydrolysis products of UF_6 at an air concentration of 726± 289 mg U m^{-3}, with a particle size of 1.07 μm MMAD (σ_g = 1.73).

NO. OF RATS	INITIAL BODY WEIGHT IN g		POSTEXPOSURE TIME IN DAYS	INHALED DOSE, mg U kg^{-1}		ABSORBED DOSE, mg U kg^{-1}		MEAN PERCENT OF ABSORBED DOSE OF U			
	MEAN	SDEV		MEAN	SDEV	MEAN	SDEV	URINE	BONE	KIDNEYS	LUNGS
4	267	23	0.1	8.9	4.4	3.4	1.3		72	14	13
4	263	35	1	9.1	1.9	3.7	1.0	18	56	21	5
4	264	56	2	9.5	2.2	3.8	1.4	25	48	22	5
5	261	15	3	9.0	1.3	3.6	0.5	32	43	21	4
4	243	51	4	8.4	4.0	2.8	1.9	36	43	18	4
4	247	32	5	7.8	0.6	2.0	0.7	43	41	11	5
4	245	22	6	8.2	0.6	2.2	0.2	39	46	13	3
3	285	6	7	8.9	2.5	3.2	0.3	41	44	12	3
1	293		9	8.4		4.2		42	41	14	3
2	272	38	38	7.2	1.2	1.7	0.8	62	29	4	6

Table 10. Experiment No. 14. The inhaled dose of U, absorbed dose and distribution of absorbed dose in LE rats exposed one hr to the hydrolysis products of UF_6 at an air concentration of 816±98 mg U m^{-3}, with a particle size of 1.80 µm MMAD (σ_g = 1.61).

of the UO_2F_2 aerosol varied by as much as a factor of two. For example, the range of mean values for urine was 52 to 56 percent, for bone 36 to 39 percent, for lungs 4 to 5 percent and for kidneys 3 to 5 percent at two weeks post-exposure (see Tables 7, 8 and 9).

Cumulative U excretion curves obtained from each of the first four inhalation experiments are plotted in Figures 12-15. A reasonably good correlation can be seen between total U excreted and the inhaled dose; as the inhaled dose of U increases so does the total amount of U excreted in the urine and feces. At six days post-exposure, fecal and urinary U in Experiment No. 14 (Figure 15) accounted for 82 percent of the inhaled dose (8.6 mg U kg^{-1}) compared to 75 percent of the inhaled dose (7.6 mg U kg^{-1}) in Experiment No. 11 (Figure 12), 73 percent of the inhaled dose (6.8 mg U kg^{-1}) in Experiment No. 13 (Figure 14) and 66 percent of the inhaled dose (6.2 mg U kg^{-1}) in Experiment No. 12 (Figure 13).

C. Uranium Retention

Organ retention graphs for U, obtained in Experiment No. 14, are presented in Figures 16, 17, 18 and 19. Retention values were calculated and plotted in two different ways. Figures 16, 17 and 18 present graphs of U content as a mean percent of the inhaled dose* vs. post-exposure days. In Figure 19, the mean percent of the absorbed dose** is plotted against post-exposure time.

From the data obtained in Experiment No. 14, the U retention equations for kidneys and bone covering the 3 to 38 day post-exposure period were computed to be:

$$R_{bone} = 38.84e^{-0.01t} \qquad R^2 = 0.91$$
$$R_{kidneys} = 18.22e^{-0.04t} \qquad R^2 = 0.87$$

thus giving biologic half times of 69.3 days for bone and 17.3 days for kidneys.

The relation between absorbed dose and inhaled dose of U found in Experiment No. 14 is presented graphically in Figure 20. According to this figure, the ratio of absorbed dose to inhaled dose averaged

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*The inhaled dose can be defined as that amount of U that enters the body through the respiratory system and is ultimately absorbed into the body or excreted. The inhaled dose is the sum of the U contents of the urine, feces, bone, kidneys, lungs and gastrointestinal tract (GIT).

**The absorbed dose can be defined as that part of the inhaled dose of U which comes into direct contact with the blood, is promptly translocated to bone and kidneys and ultimately excreted in the urine. Thus, in these rodent studies the absorbed dose is the sum of the U contents of the urine, bone, kidneys and lungs.

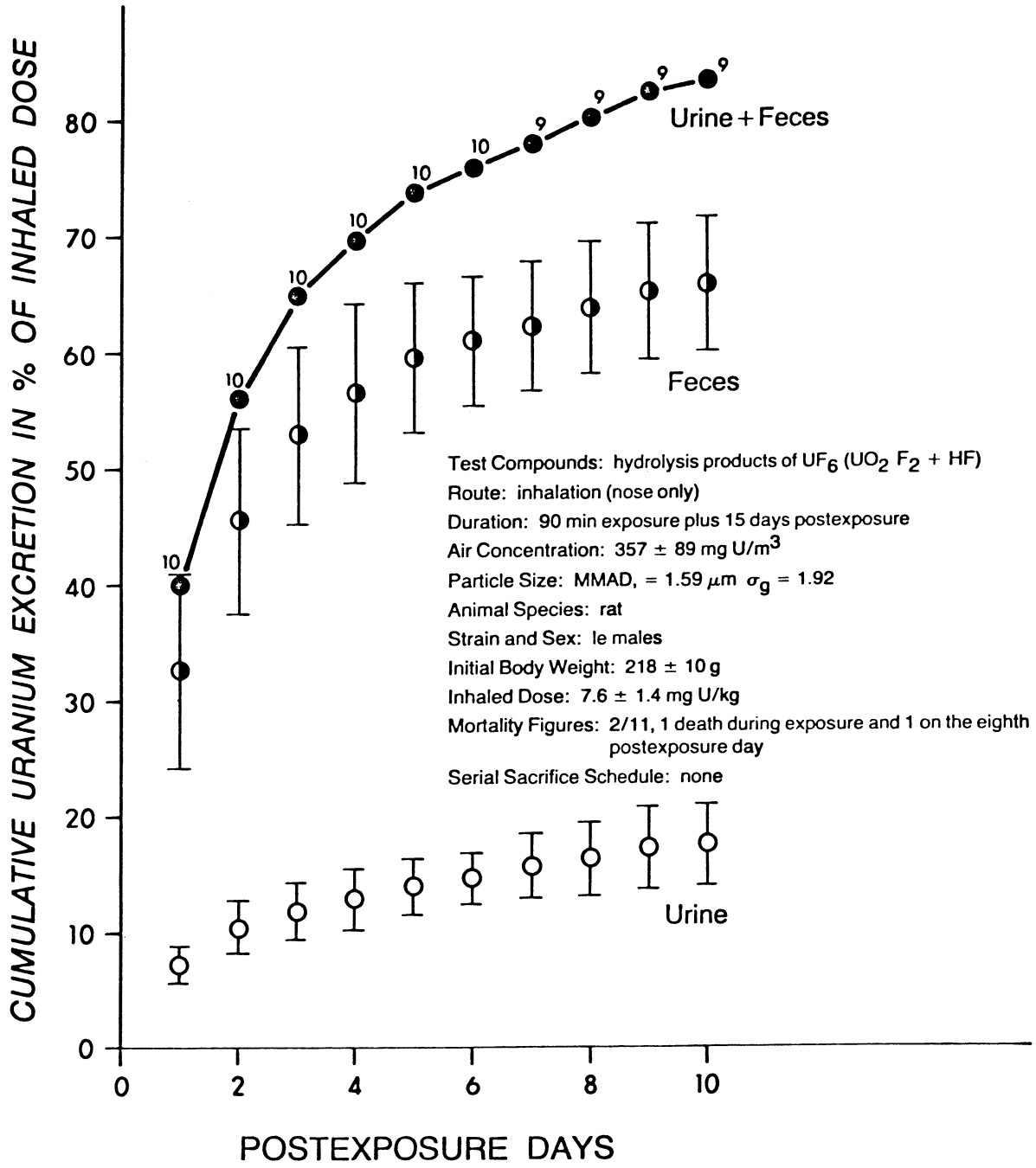

Figure 12. Inhalation Experiment No. 11

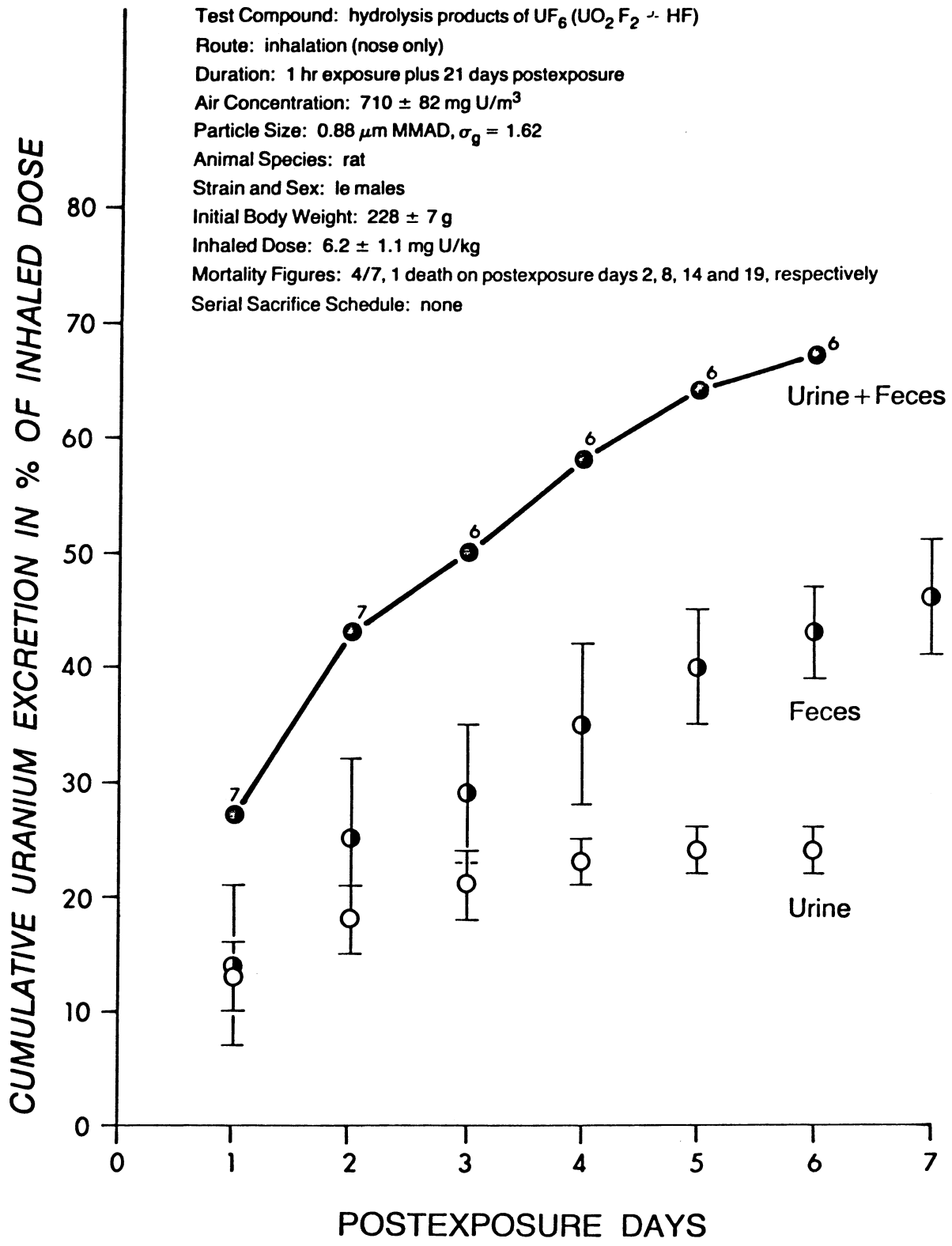

Figure 13. Inhalation Experiment No. 12

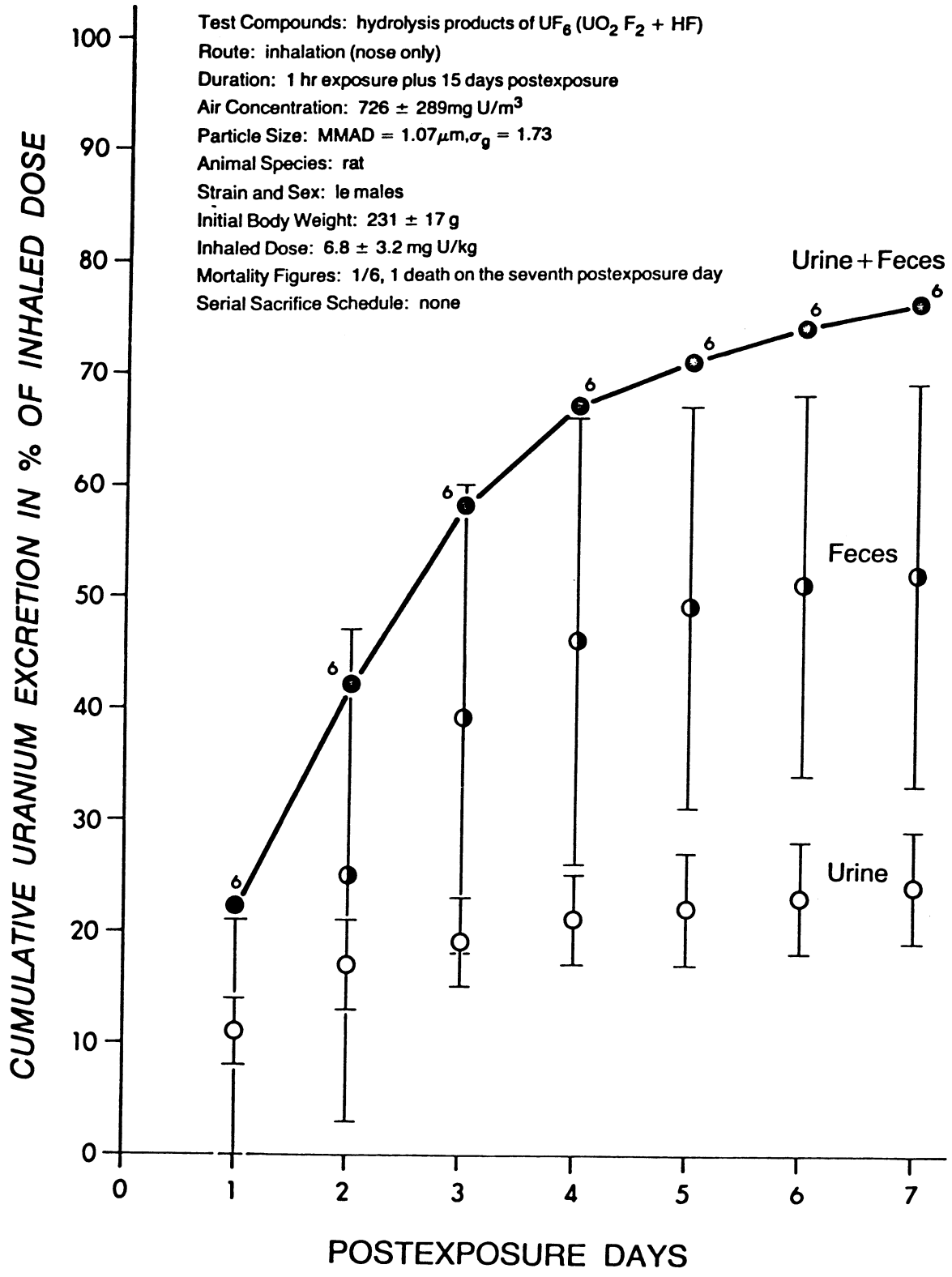

Figure 14. Inhalation Experiment No. 13

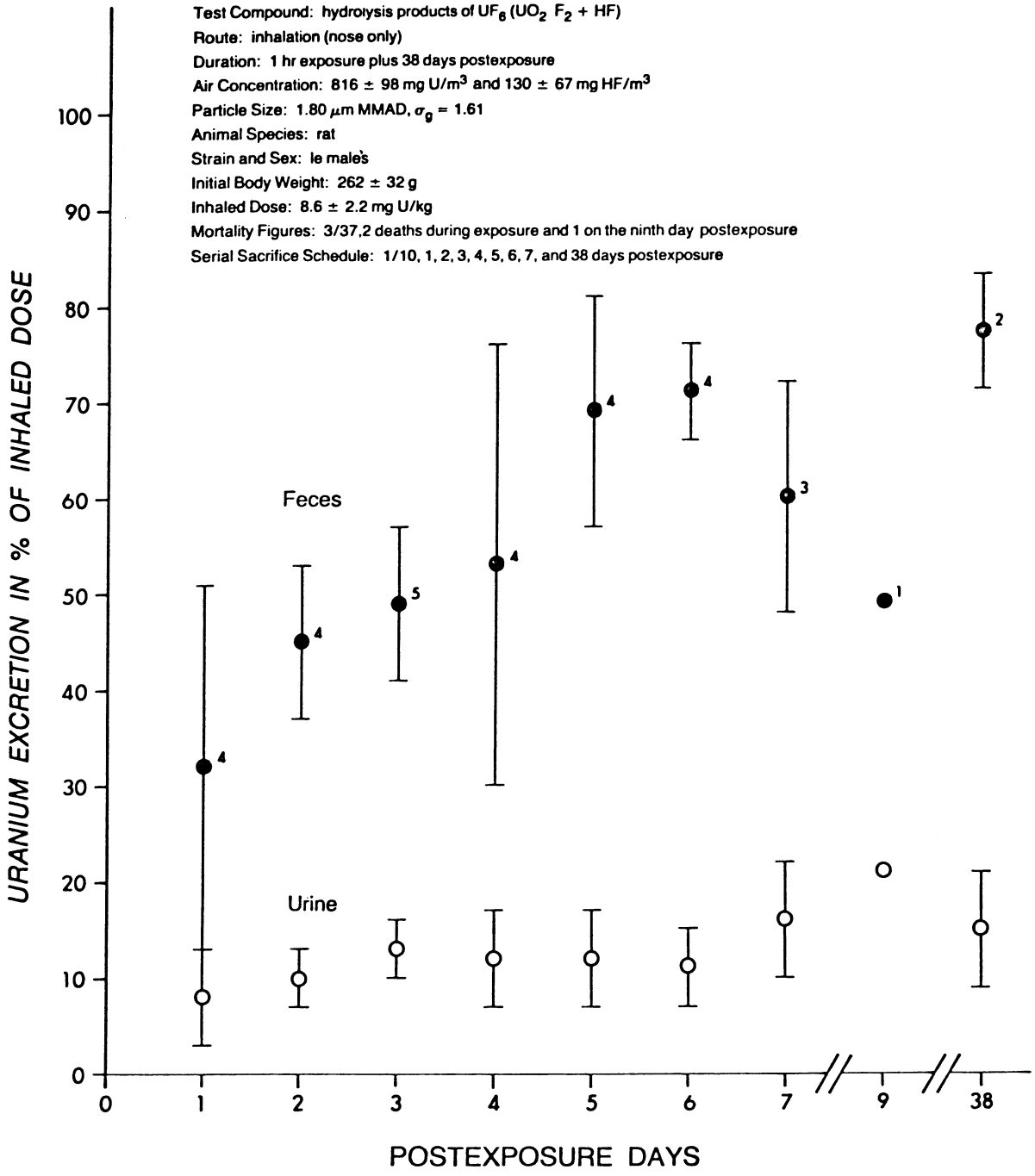

Figure 15. Inhalation Experiment No. 14

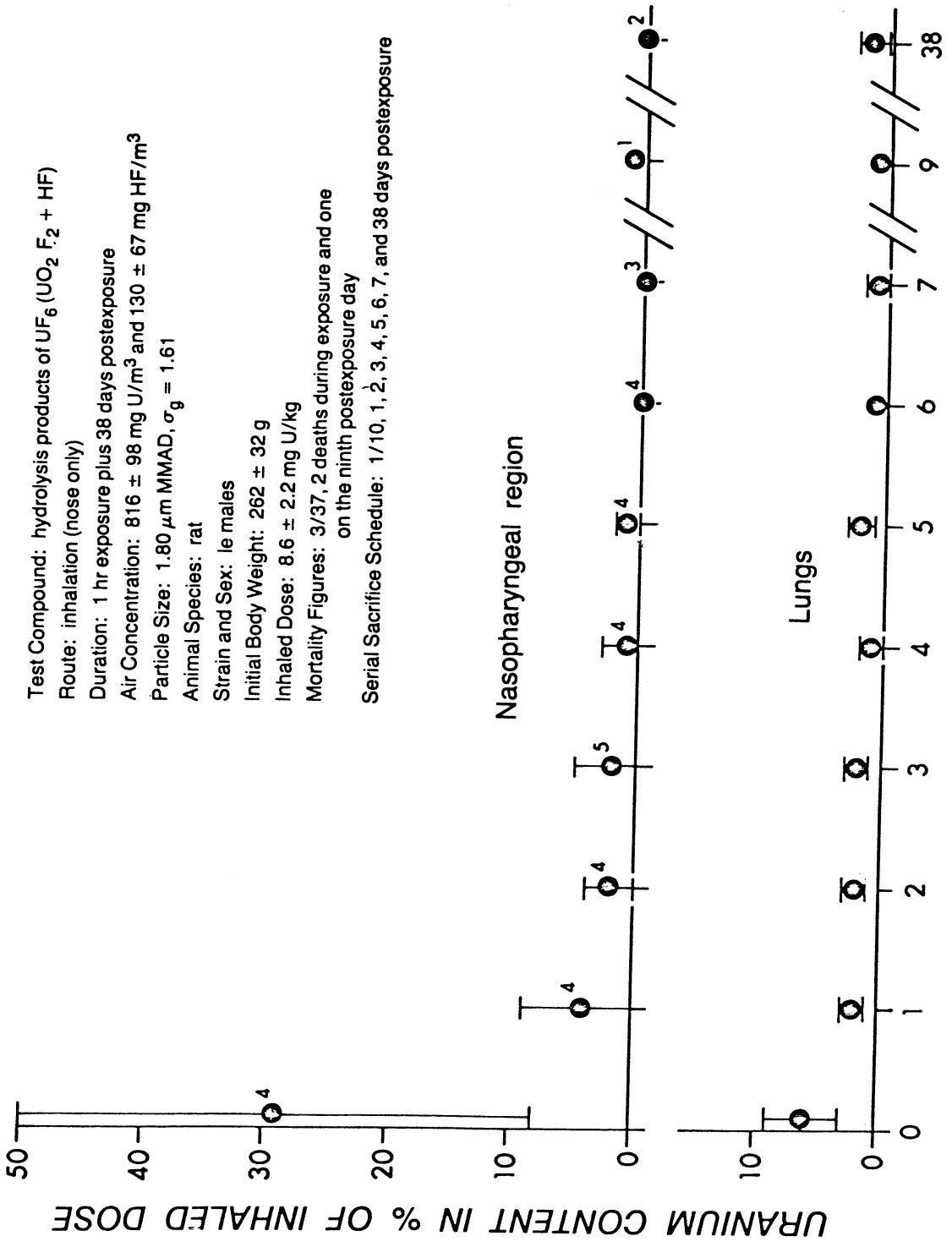

Figure 16. Inhalation Experiment No. 14

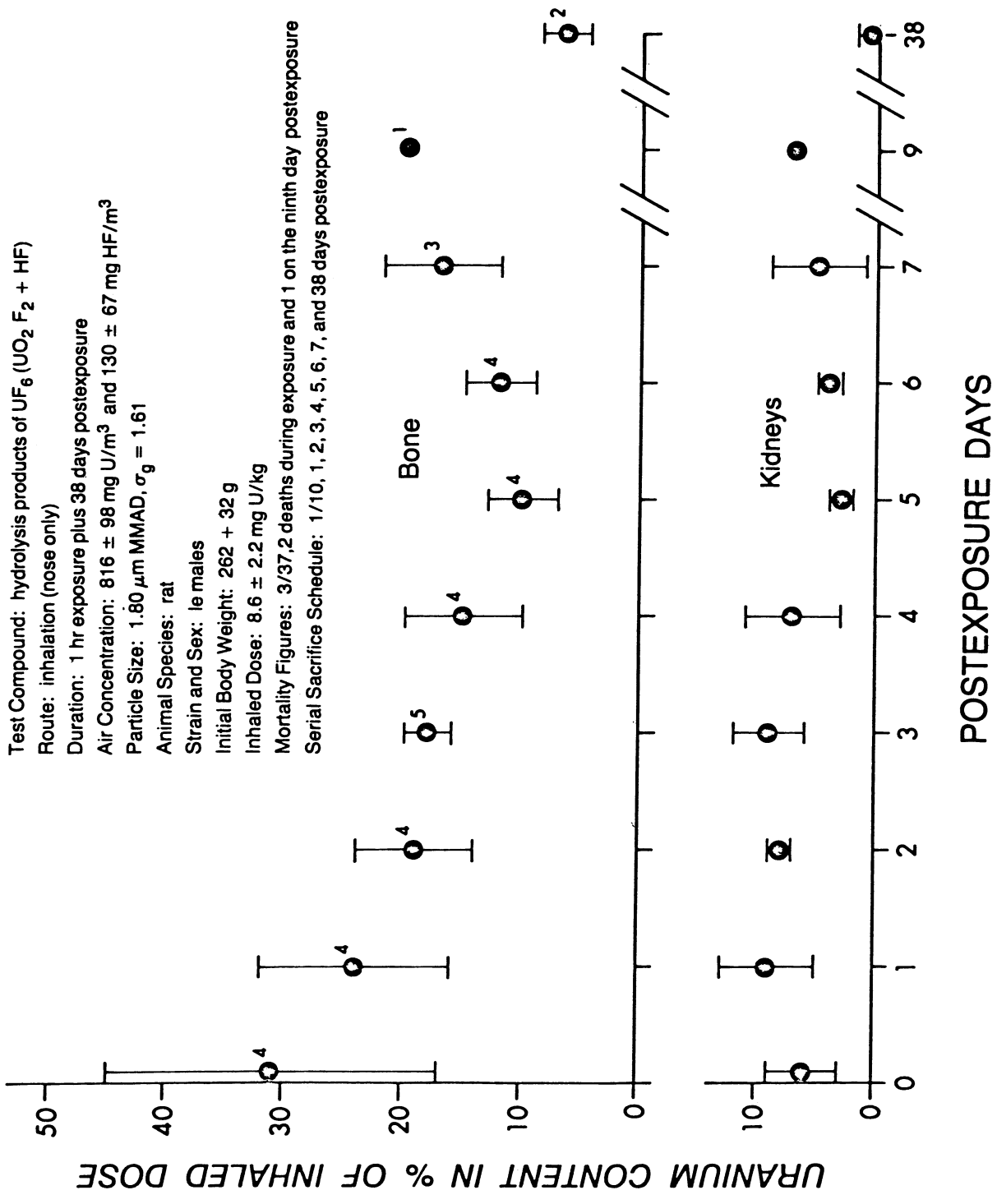

Figure 17. Inhalation Experiment No. 14

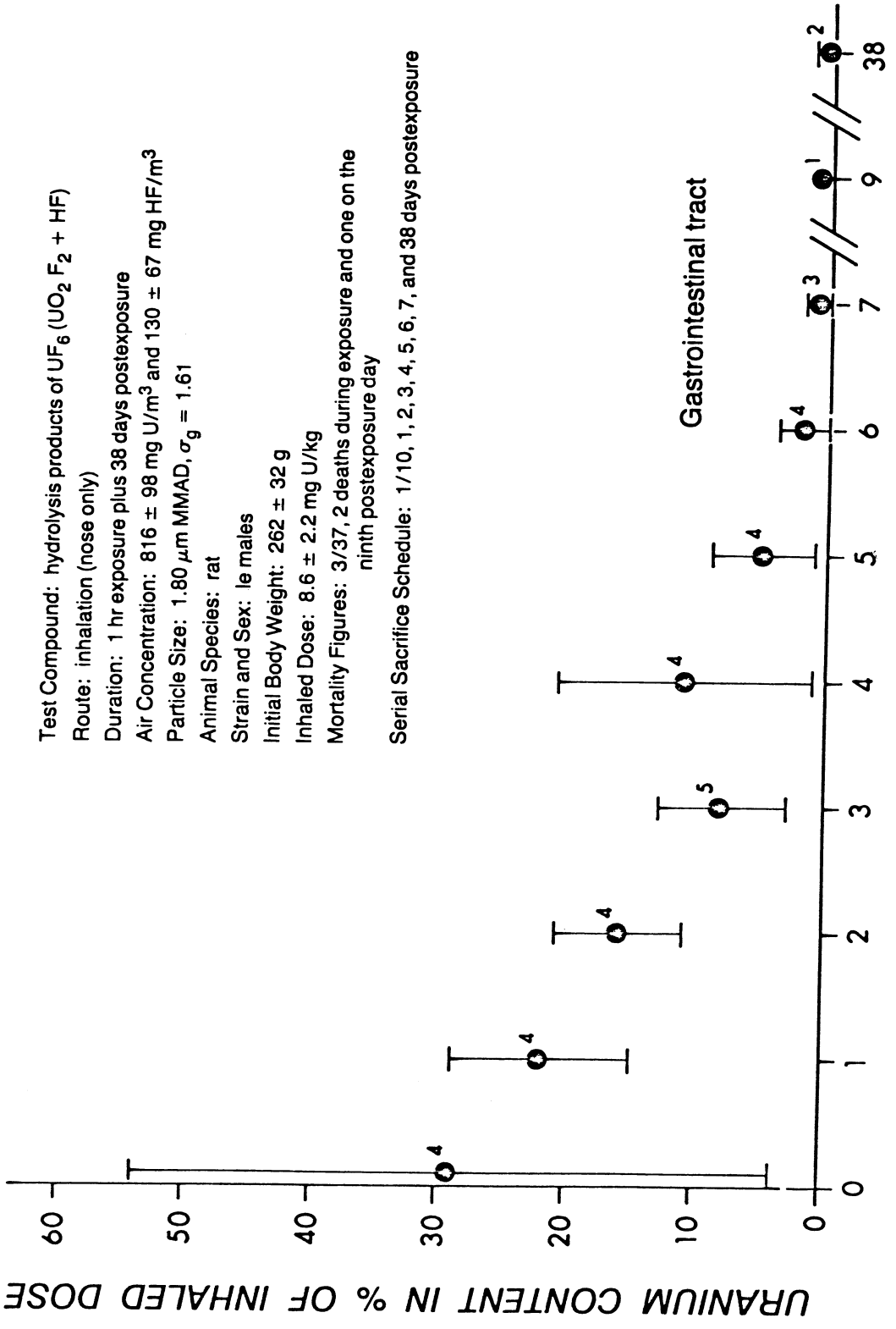

Figure 18. Inhalation Experiment No. 14

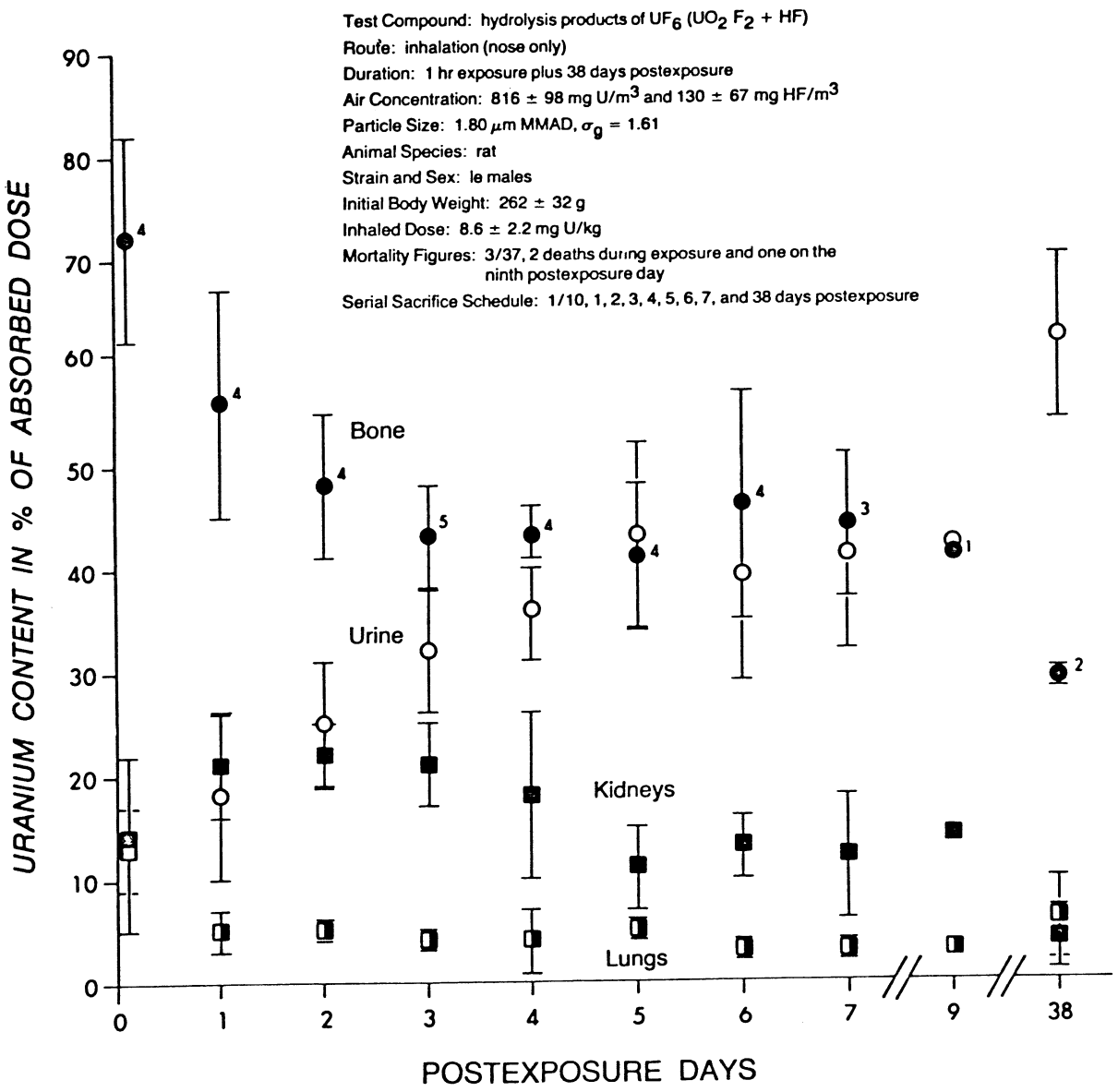

Figure 19. Inhalation Experiment No. 14

0.36, thus indicating that 64 percent of the inhaled dose of UO_2F_2 aerosol passed through the gastrointestinal tract (GIT) unabsorbed and was eventually recovered in the feces. For comparative purposes the values obtained for the ratio of absorbed dose of U to inhaled dose for Experiments 11-14 are recorded in Table 11. These ratios range from 0.33 for Experiment No. 11 to 0.53 for Experiment No. 12 and show a general increase as particle size (MMAD) of the aerosol decreases.

D. Assessment of Renal Injury (Inhalation Studies)

Experiment No. 15 (Table 6) was a one hour nose-only inhalation exposure of eight rats to natural UF_6 hydrolysis products (UO_2F_2 + HF) at a U concentration level of 284 + 39 mg m^{-3}. Although U excretion and retention were not followed in this experiment (because μg quantities of natural U are not easily measured using gamma counting techniques), we estimate from previous experience that the absorbed dose in these animals was of the order of 2 to 3 mg U kg^{-1}.

To assess renal injury associated with exposure to U^{+6} (UO_2F_2) and possibly F (HF) the following criteria were investigated in survivors over a two week post-exposure period: water consumption, urinalyses (volume, protein, glucose, amino acids, fluoride and phosphate), renal function (creatinine clearance and plasma urea nitrogen concentrations) and levels of activity of a urinary enzyme, N-acetyl-β-glucosaminidase (NAG). The results of these assessments are presented graphically in Figures 21 through 25. These graphs illustrate the typical biochemical and physiological changes observed when rats were exposed to non-lethal but nephrotoxic levels of U, that is, more than 0.1 mg U^{+6} kg^{-1} but less than 3 mg U^{+6} kg^{-1}, as absorbed dose.

Figure 21 indicates that diuresis developed about three days after exposure to hydrolyzed natural UF_6. Water consumption more than doubled and urine output increased to five times control values by the seventh post-exposure day. Although the daily water consumption and urine volume declined during the following week, both volumes were still higher than control values on the fourteenth post-exposure day.

The daily post-exposure urinary excretion of NAG (Figure 22) increased after the first post-exposure day and reached maximum values on the third and fourth post-exposure day. A second peak for urine NAG excretion occurred during the middle of the second week post-exposure; this appears to be a typical response (see Experiment No. 11) but its meaning is unknown at this time. Maximum proteinuria occurred on the fourth post-exposure day. Two weeks after exposure to hydrolyzed UF_6, urinary protein and NAG levels had returned to somewhat higher than normal (1.5 to 2 times) values.

There was a marked elevation in urine glucose and amino acids beginning on the third post-exposure day (Figure 23). Both curves

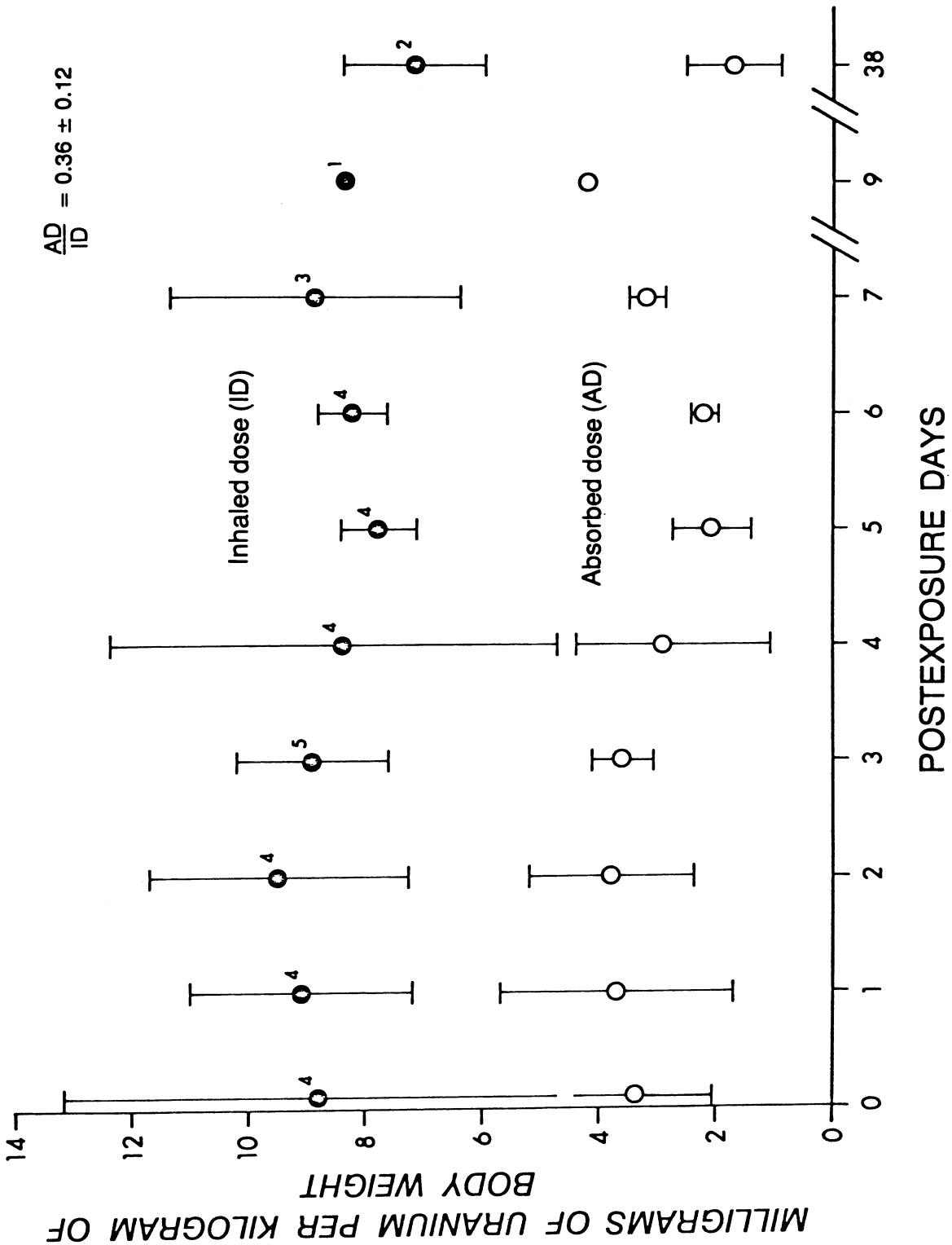

Figure 20. Inhalation Experiment No. 14

EXPERIMENT NUMBER	PARTICLE SIZE DATA		ABSORBED DOSE	UO_2F_2 AEROSOL	EXPOSURE
	MMAD, μm	σ_g	INHALED DOSE	CONC., mg U/m^{-3}	TIME, hr
11	1.59	1.92	0.33 ± 0.06	357 ± 89	1.5
14	1.80	1.61	0.36 ± 0.12	816 ± 98	1
13	1.07	1.73	0.41 ± 0.06	726 ± 289	1
12	0.88	1.62	0.53 ± 0.04	710 ± 82	1

Table 11. The relationship between absorbed dose and inhaled dose of U in four experiments where rats inhaled hydrolyzed UF_6 (FE).

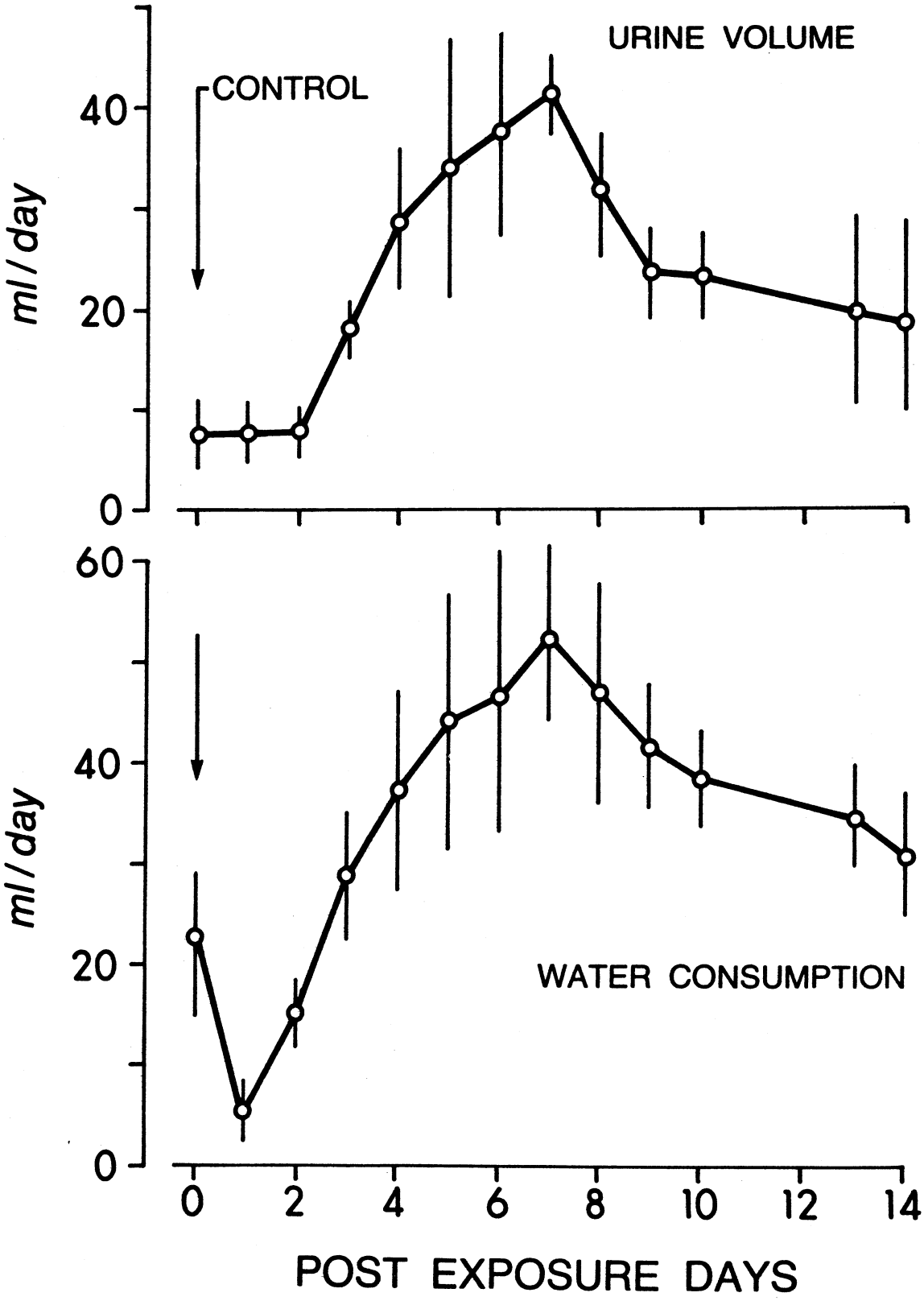

Fig. 21 Urine output (top), Water consumption (bottom). For Inhalation Experiment No. 15. The results are means ± S.D. from 7 rats.

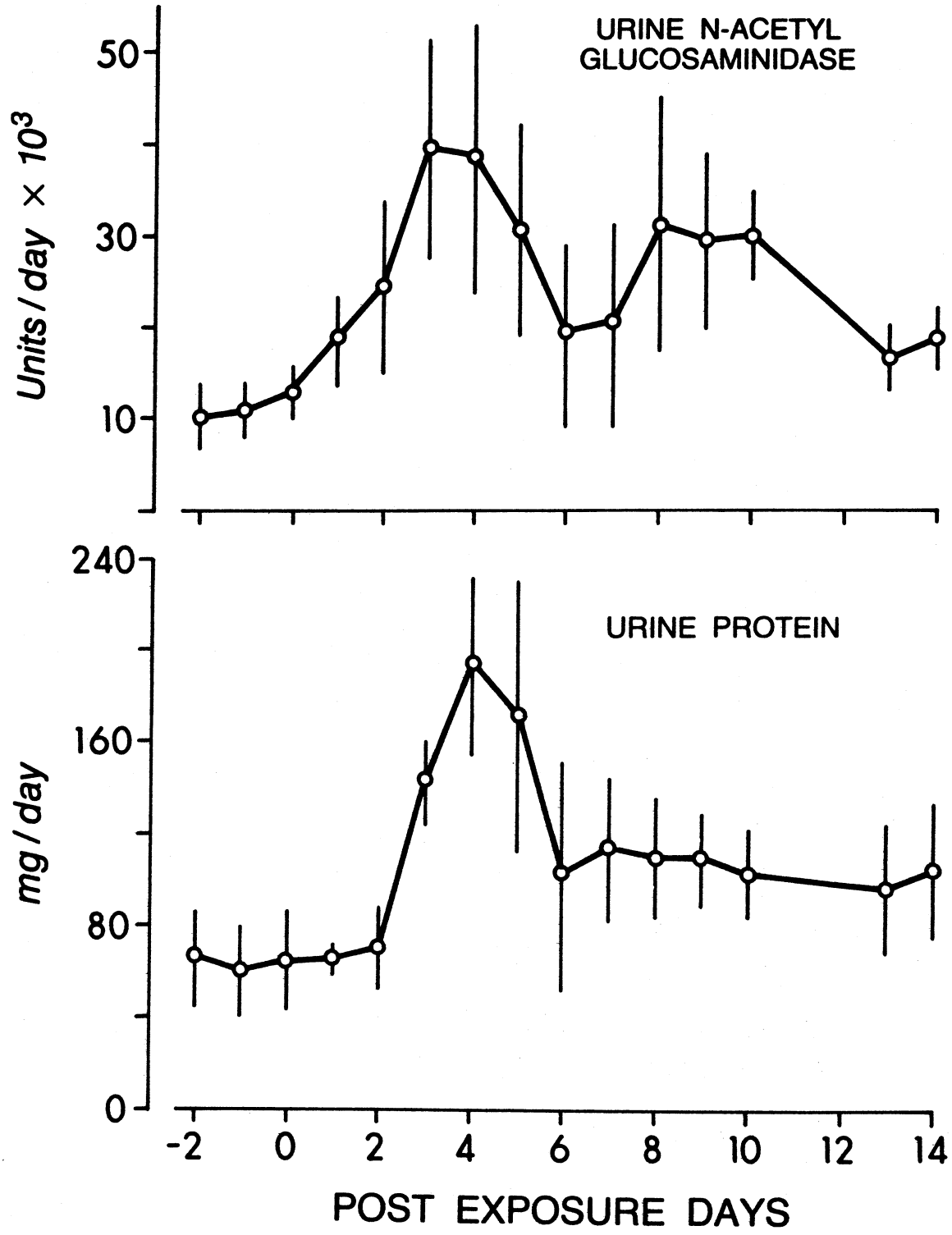

Fig. 22 Urine N-acetyl-β-glucosaminidase activity (top), Urine protein (bottom). For Inhalation Experiment No. 15. The results are means ± S.D. from 7 rats.

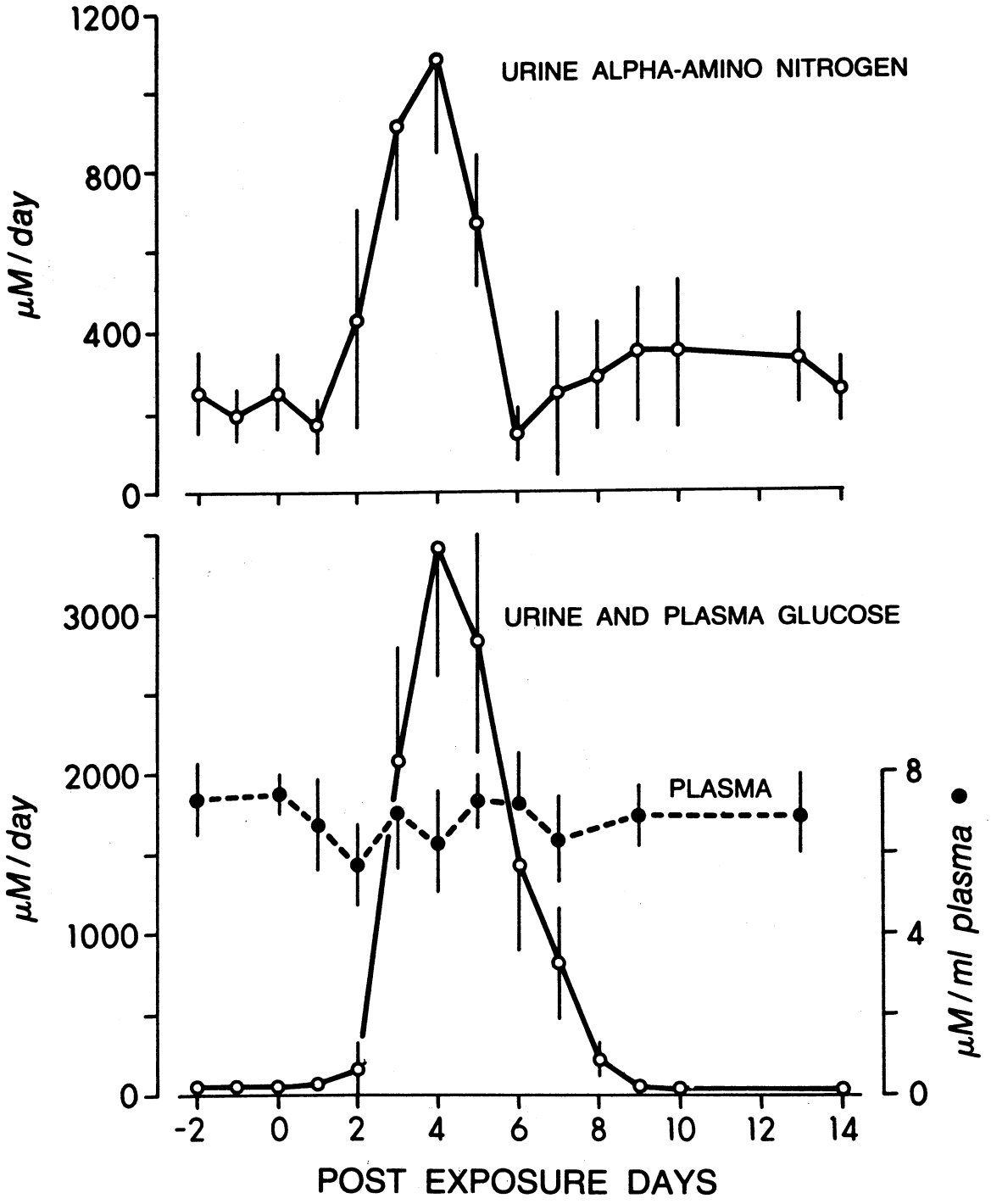

Fig. 23 Urine alpha-amino nitrogen (top), Urine and plasma glucose (bottom). For Inhalation Experiment No. 15. The results are means ± S.D. from 7 rats.

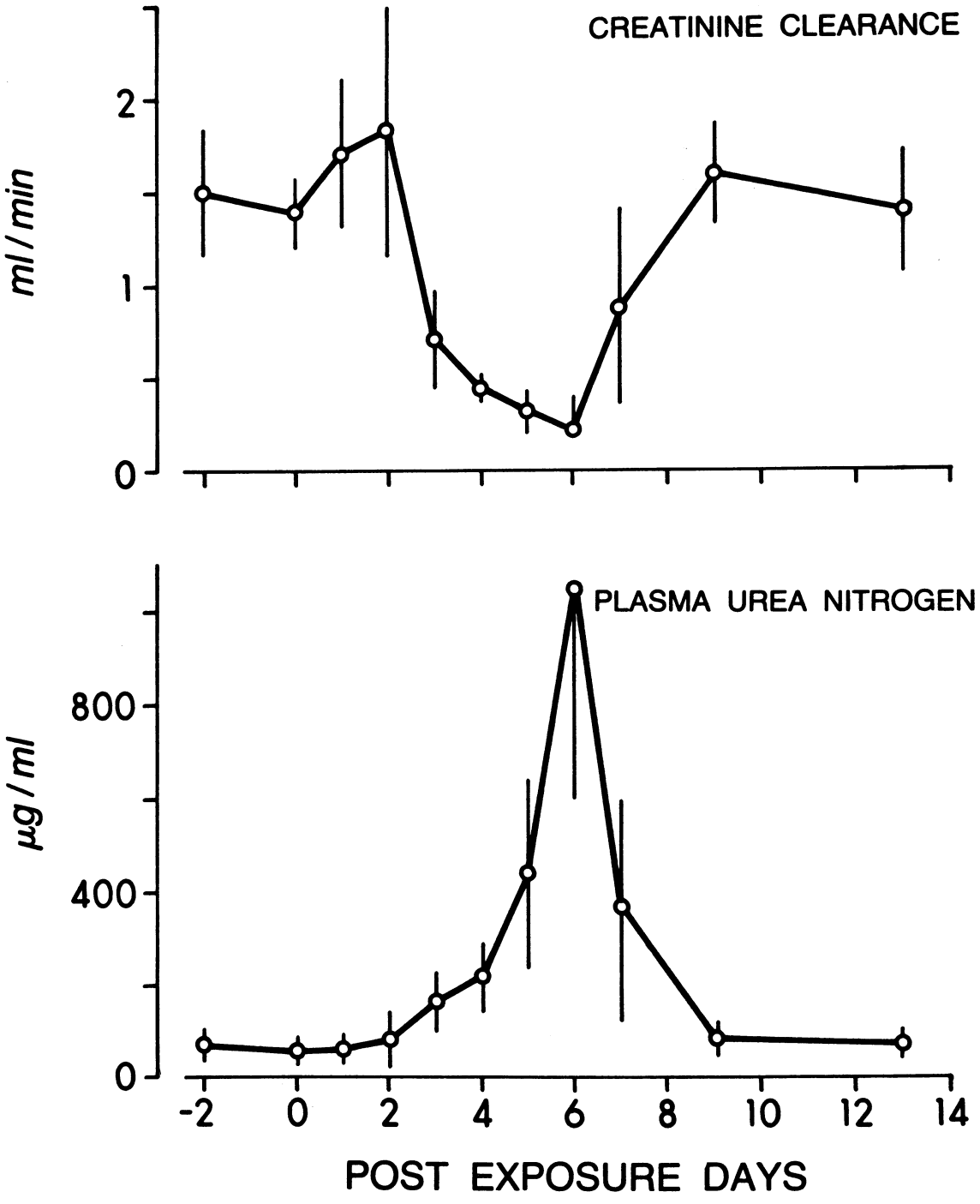

Fig. 24 Creatinine clearance (top) and Plasma urea nitrogen (bottom). Levels for Inhalation Experiment No 15. The results are means ± S.D. from 7 rats.

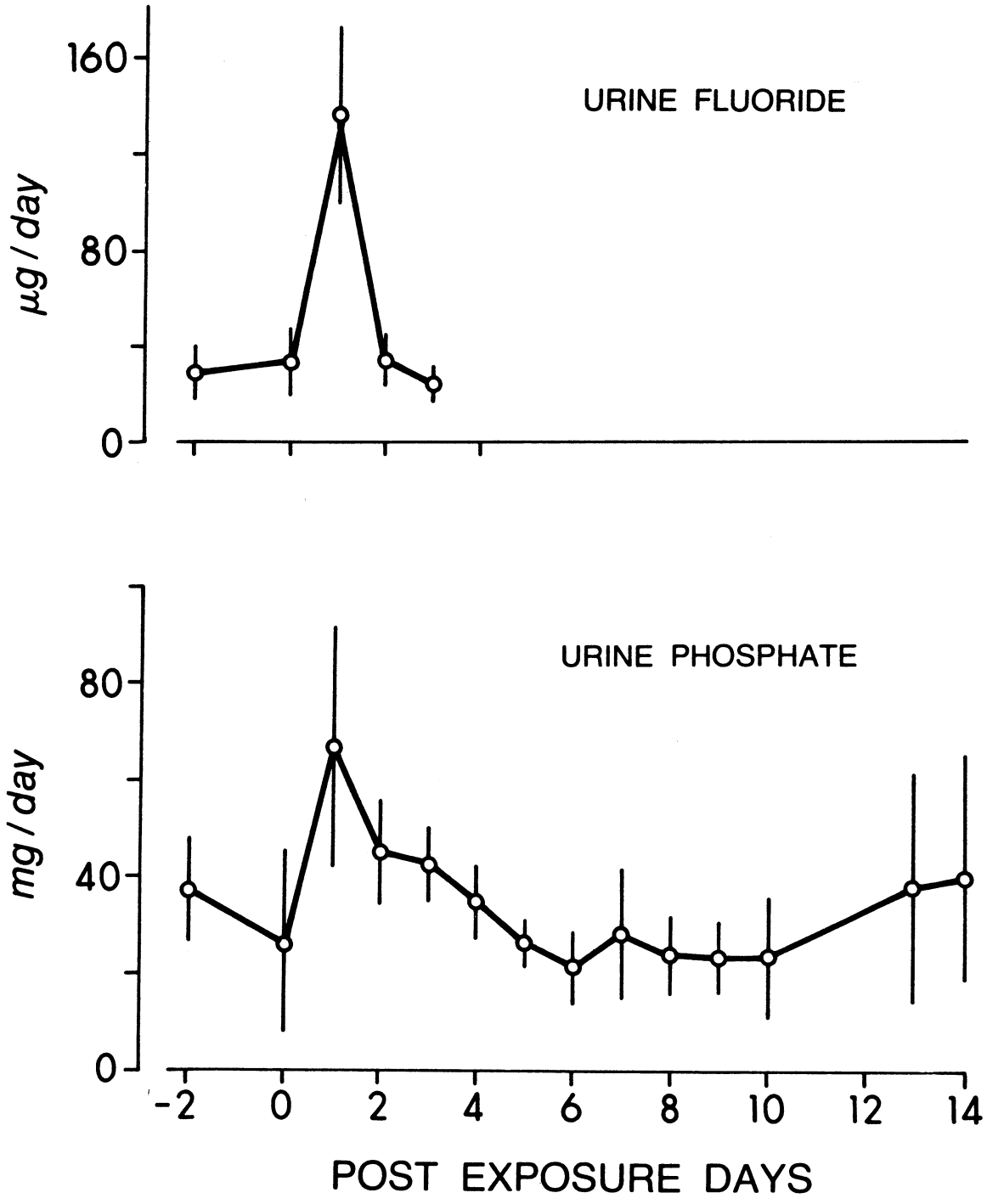

Fig. 25 Urine fluoride (top) and phosphate (bottom). For Inhalation Experiment No. 15. The results are means ± S.D. from 7 rats.

peaked at the fourth day after exposure and subsequently returned to pre-exposure levels. The large increase in urine glucose (150-200 times) appears to be unrelated to any changes in plasma glucose levels (see Figure 23).

Creatinine clearance was used in these studies as an estimate of glomerular filtration rate. Figure 24 (top) shows the rapid fall of the creatinine clearance rate on the third post-exposure day and after reaching a minimum value on the sixth day. The rate returned to normal by the ninth day. Similarly, the plasma urea concentration reflected a transient fall in urea clearance over the same post-exposure time period (Figure 24, bottom).

Figure 25 describes the renal excretion of fluoride and phosphate in rats from Experiment No. 15. Fluoride levels in the urine rose sharply during the first day after exposure and returned rapidly to control levels (see Figure 25, top). The purpose of following fluoride in the urine was to determine if fluoride excretion could be used to monitor exposure to UO_2F_2 and HF. This assay was discontinued because there were too many uncontrollable sources of fluoride entering the rat through the food and water. Phosphate measurements in the urine were found to be a poor and insensitive indicator of renal injury in rats exposed to hydrolyzed UF_6; this is graphically demonstrated in Figure 25 and also in Table 12.

An almost identical pattern of renal injury followed exposure of rats to the hydrolysis products of enriched UF_6 (Experiment No. 11), as demonstrated in Table 12. These data can be directly compared with Experiment No. 15 since the only major difference was the use of enriched instead of natural uranium.

Urinalyses indicated renal intoxication by persistent, increased urine volume, proteinuria (from 3 to 7 days post-exposure), glucosuria (from 2 to 7 days post-exposure), aminoaciduria (2 to 7 days post-exposure), and bimodal NAG enzymuria (1 to 5 days and 8 to 15 days post-exposure). As can be seen in Table 12, plasma urea nitrogen increased to a maximum value approximating 1300 μg/ml at six days post-exposure and then returned to normal during the following week.

When the data from Experiments No. 15 and 11 are compared, it would appear that the radioactivity associated with enriched UF_6 (Experiment No. 11) had no unique effect on rat kidneys that was measurable by our renal injury indicators.

E. Multiple Dose Intravenous Studies

Experiments No. 16, 17 and 18 (see Table 6) were undertaken to provide information on the value of various nephrotoxic indicators when used on animals that received multiple subliminal doses (0.1 mg U kg^{-1}) or multiple non-lethal but nephrotoxic doses (1 mg U kg^{-1}) prior to a test dose of 5 mg U kg^{-1}. The dose and time between injections was chosen from experience gained from other

POSTEXP (days)	URINE VOL. ml/day)	URINE PROTEIN (mg/day)	URINE NAG (units/day)	URINE GLUCOSE (μM/day)	URINE AAN (μM/day)	URINE PO_4 (mg/day)	PLASMA UN (μg/ml)
Control	9 ± 2	75 ± 16	6,621 ± 3,328	30 ± 16	216 ± 36	30 ± 10	141 ± 22
1	10 ± 7	53 ± 10	10,995 ± 3,328	51 ± 49	112 ± 22	64 ± 6	152 ± 47
2	9 ± 4	97 ± 26	25,479 ± 9,351	528 ± 375	528 ± 279	59 ± 6	361 ± 136
3	20 ± 8	144 ± 53	37,885 ± 15,119	2,476 ± 1,091	1,530 ± 635	46 ± 11	360 ± 73
4	32 ± 8	155 ± 16	18,604 ± 4,590	2,380 ± 447	1,930 ± 301	33 ± 11	614 ± 149
5	37 ± 7	151 ± 51	13,128 ± 4,745	1,578 ± 115	1,301 ± 454	31 ± 15	1,259 ± 113
6	25 ± 9	138 ± 45	7,457 ± 5,331	682 ± 424	624 ± 277	16 ± 10	938 ± 113
7	31 ± 14	128 ± 46	8,715 ± 4,430	691 ± 429	744 ± 331	19 ± 9	938 ± 259
8	33 ± 10	100 ± 36	13,914 ± 5,108	212 ± 220	332 ± 180	21 ± 9	----
9	28 ± 12	88 ± 24	22,627 ± 12,357	158 ± 164	932 ± 365	12 ± 7	519 ± 205
13	19 ± 5	93 ± 13	23,282 ± 23,222	23 ± 20	758 ± 224	15 ± 11	159 ± 26
15	20 ± 6	94 ± 33	13,575 ± 5,683	12 ± 11	510 ± 209	28 ± 19	182 ± 35

Results are means ± S.D. of 8 to 10 rats

Table 12. Summary data from 90 minute exposure (Experiment No. 11) to UF_6 hydrolysis products at an air concentration of 357±89 mg U/m^3. The mean absorbed dose was 2.47±0.39 mg U/kg

studies. A dose of 0.1 mg U kg^{-1} is not likely to produce any significant proteinuria in our rats (NUREG/CR-1045, Morrow et al., 1980). The time interval of six days between injections was chosen because this was the time needed for maximum reduction in glomerular filtration rate (see Figure 24).

The responses of the various indicators of renal injury to these dose protocols are shown in Figures 26 through 34. Growth curves shown in Figure 26, indicate a slight depression in growth rate in animals receiving intravenous U^{+6}. Although the water consumption of UO_2F_2 treated rats was near normal, the mean 24 hour urine volumes tended to be higher than control values (see Figure 27).

Our normal rats excrete 50 to 100 mg of protein per day in the urine. The average excretion of protein in animals dosed with 0.1 mg U kg^{-1} never exceeded 100 mg day^{-1} (Figure 28). Response to the test dose of 5 mg U kg^{-1} will be discussed later.

The daily mean NAG excretion exhibited a rather large variation, but tended to be higher in rats dosed with UO_2F_2 after about the fourth injection of 0.1 mg U kg^{-1} (Figure 29).

Rats showed transient glucosuria following each injection of 0.1 mg U kg^{-1}, as shown in Figure 30.

Twenty-four hour alpha-amino nitrogen excretion (used as a measure of aminoaciduria) was unaffected after the first three injections of 0.1 mg U kg^{-1}, but showed a definite elevation following injections 4, 5 and 6 (Figure 31).

Changes in urea nitrogen and creatinine concentration in the plasma were used to estimate functional changes in the glomerular filteration process in the kidneys. The creatinine clearance and plasma urea nitrogen (PUN) values remained at control levels through five injections of 0.1 mg U kg^{-1} and showed a slight decrease in the clearance of creatinine and increase in PUN following the sixth injection (Figures 32 and 33).

It has been reported by others that citrate excretion in rats increases following U^{+6} injections (Haven and Randall, 1948). A hypothesis was proposed that this increased citrate excretion had some relationship to U excretion and the development of renal "tolerance". In the present study, no increase in citrate excretion in the urine was noted following injections of aqueous UO_2F_2 (Figure 34). To the contrary, we consistently observed a definite decrease in the daily citrate excretion following exposure to sub-lethal doses of UO_2F_2. We examined the effect of UO_2F_2 on the enzymatic assay system (citrate lyase-malic dehydrogenase) used in our studies to measure urinary citrate and found no effect. We also analyzed urine samples spiked with progressively

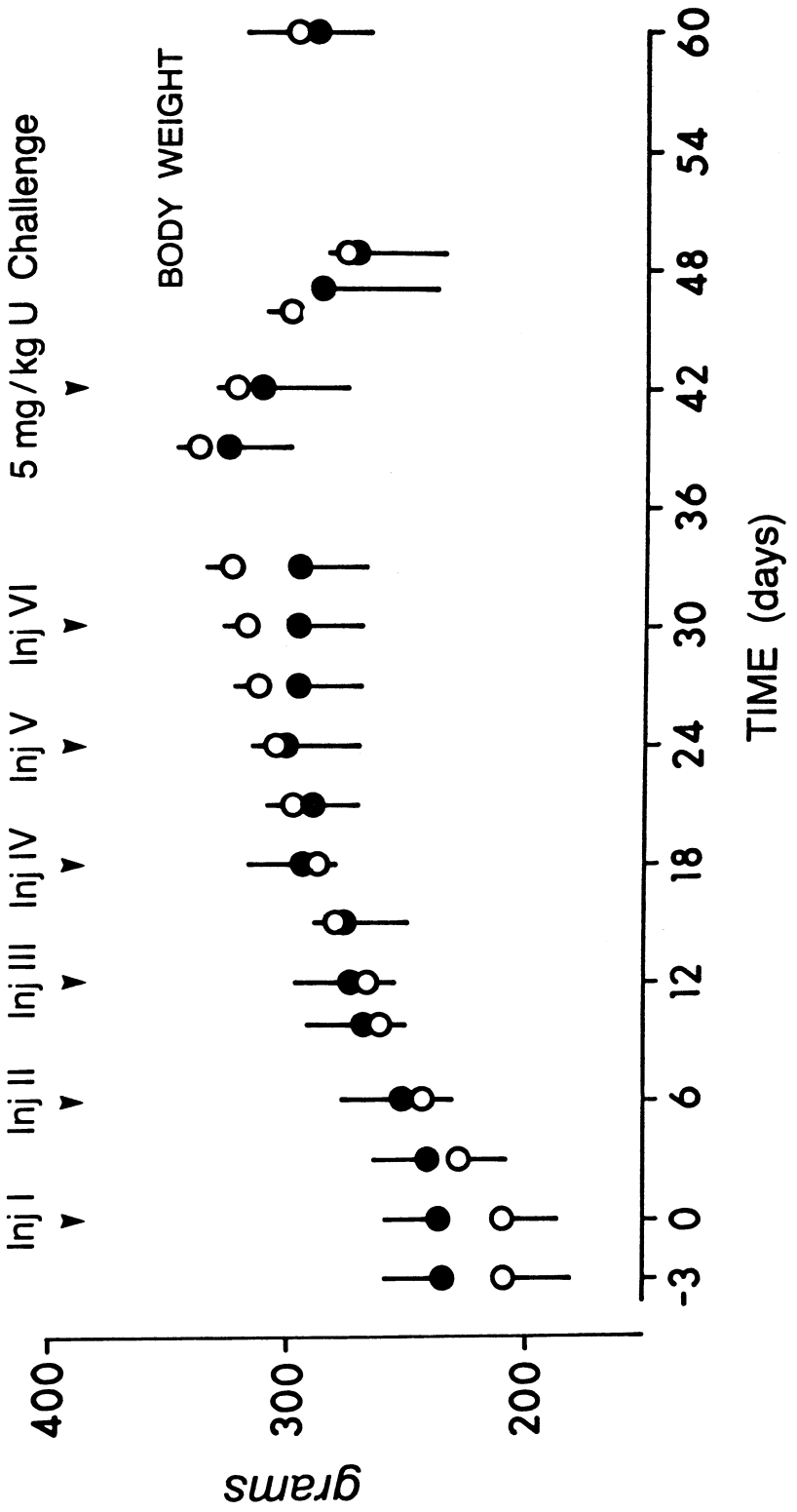

Fig. 26 The effect of multiple low doses of UO_2F_2 (6 x 0.1 mg U/kg) followed by a 5 mg U/kg challenge dose on growth rate. Solid circles are means + S.D. of six rats and open circles represent the means + S.D. of three saline controls that received the challenge dose.

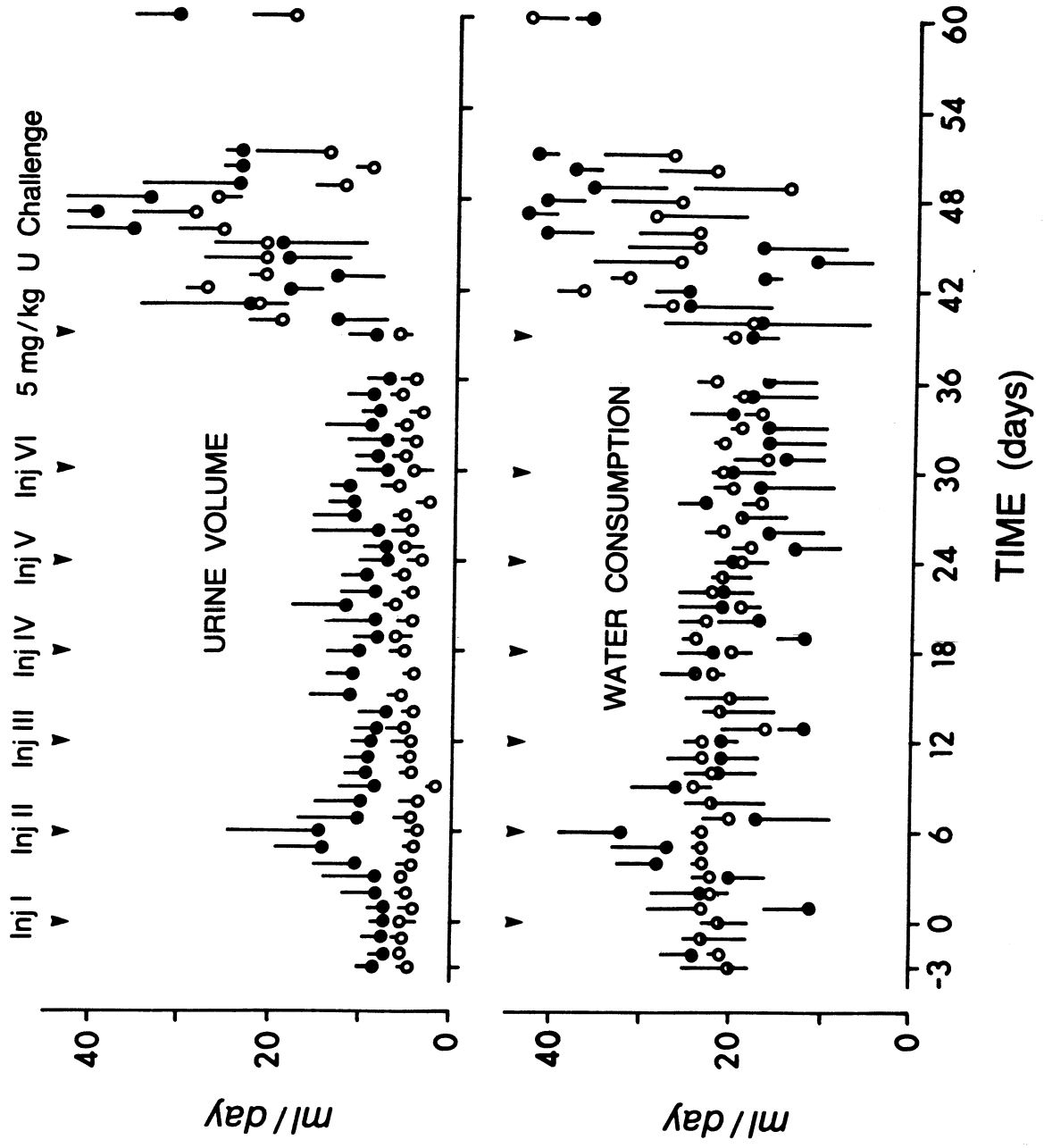

Fig. 27 The effect of multiple low doses of UO_2F_2 (6 x 0.1 mg U/kg) followed by a 5 mg U/kg challenge dose on daily urine volume (top) and water consumption (bottom). Solid circles are means ± S.D. of six rats and the open circles represent the means ± S.D. of three saline controls that received the challenge dose.

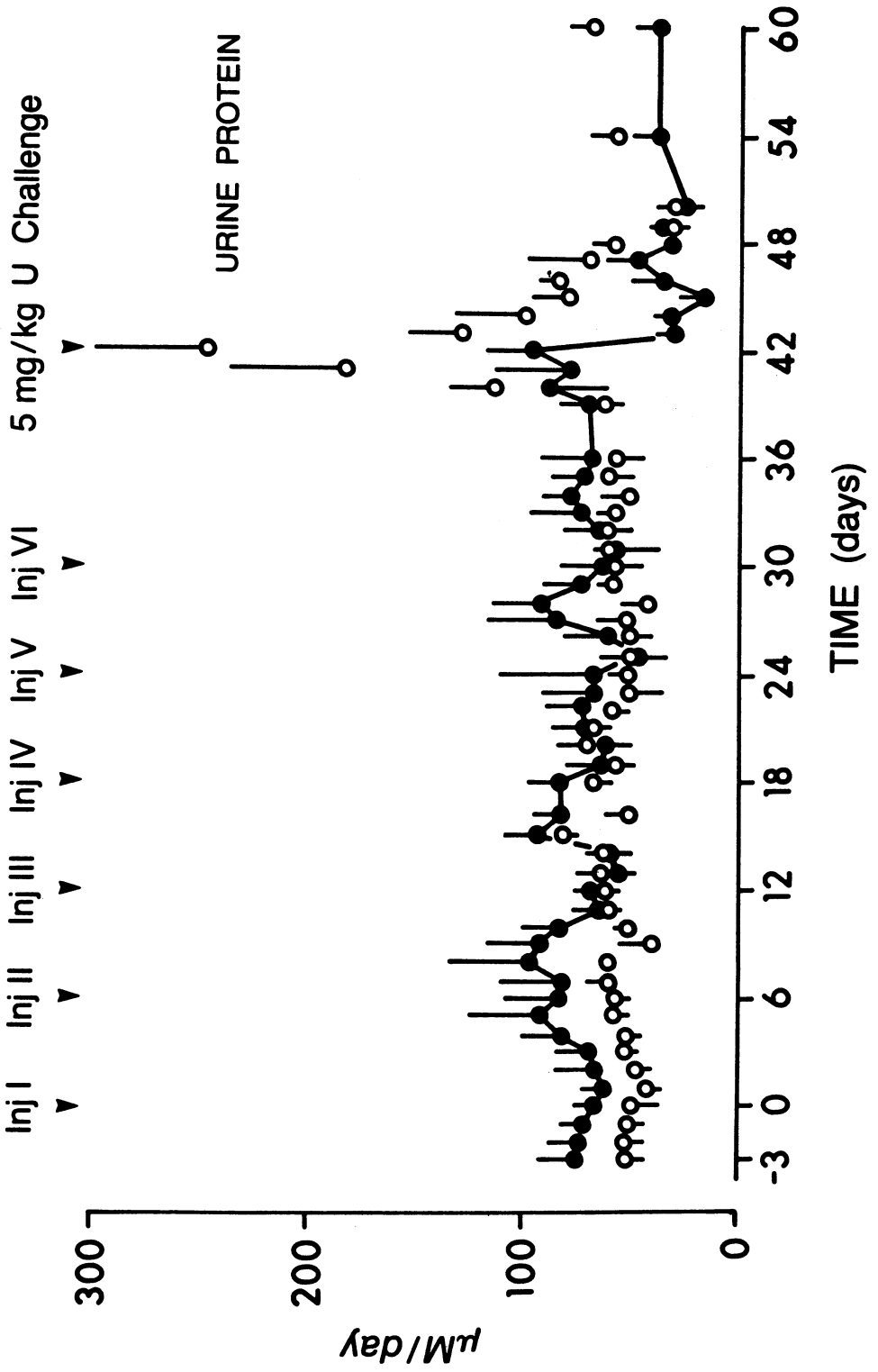

Fig. 28 The effect of multiple low doses of UO_2F_2 6 x 0.1 mg/kg) followed by a 5 mg U/kg challenge dose on daily urine protein excretion. solid circles are means $\pm$ S.D. of six rats and the open circles represent the means $\pm$ S.D. of three controls which received the challenge dose.

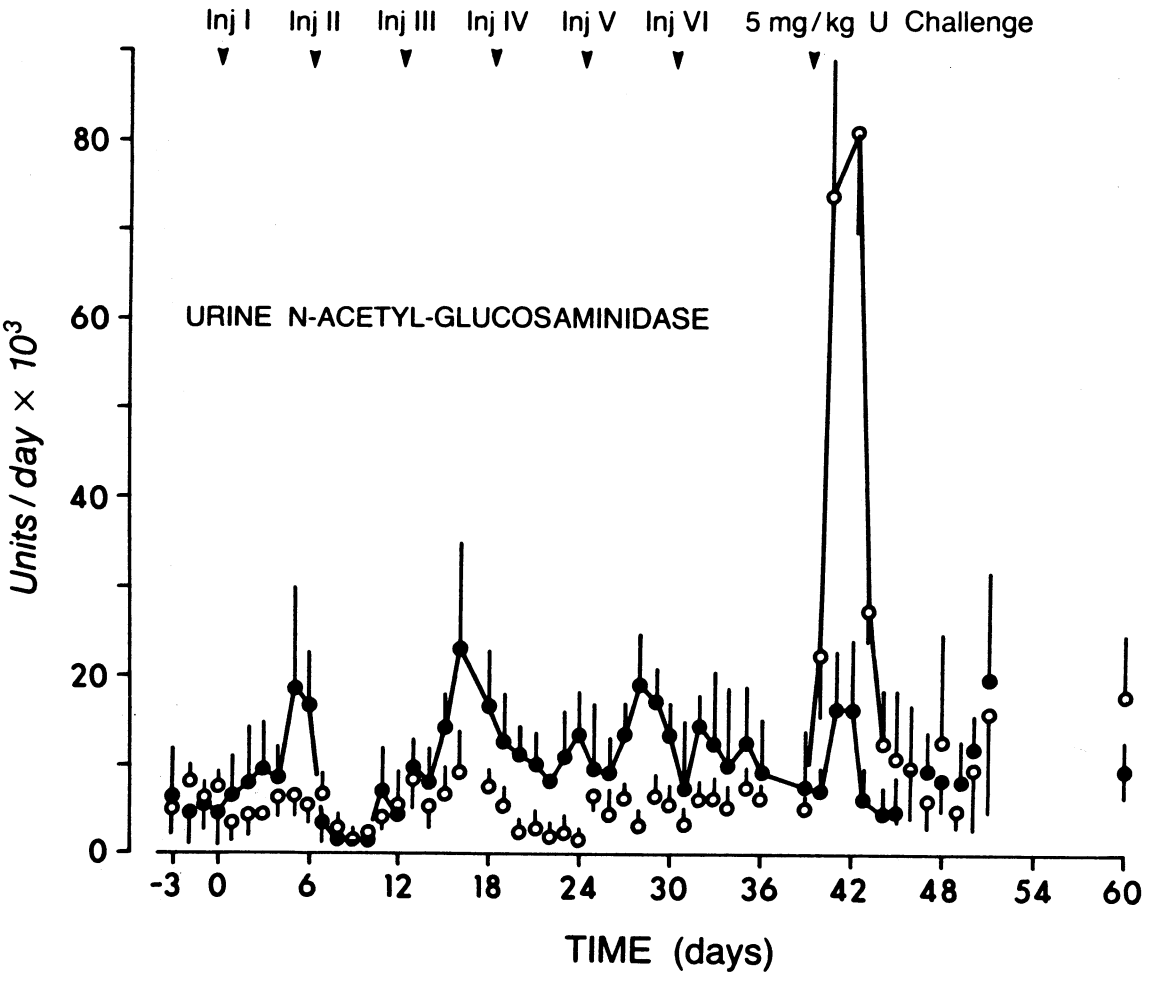

Fig. 29 he effect of multiple low doses of UO_2F_2 (6 x 0.1 mg U/kg) followed by a 5 mg U/kg challenge dose on daily excretion of N-acetyl-β- glucosaminidase. Solid circles are means ± S.D. of six rats and the open circles represent the means ± S.D. of three controls that received the challenge dose.

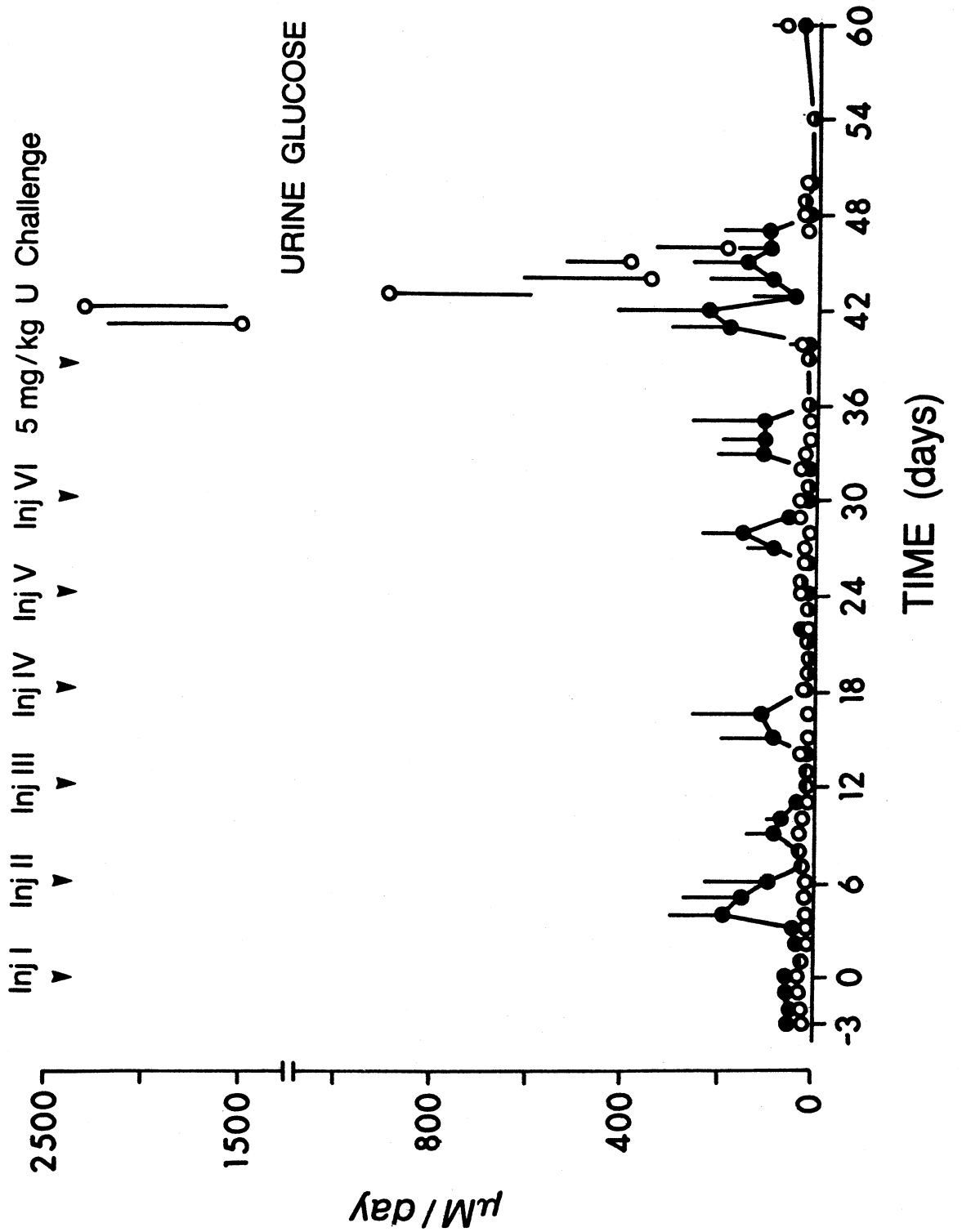

Fig. 30 The effect of multiple low doses of UO_2F_2 (6 x 0.1 mg U/kg) followed by a 5 mg U/kg challenge dose on daily urine glucose excretion. Solid circles are means ± S.D. of six rats and the open circles represent the means ± S.D. of three controls that received the challenge dose.

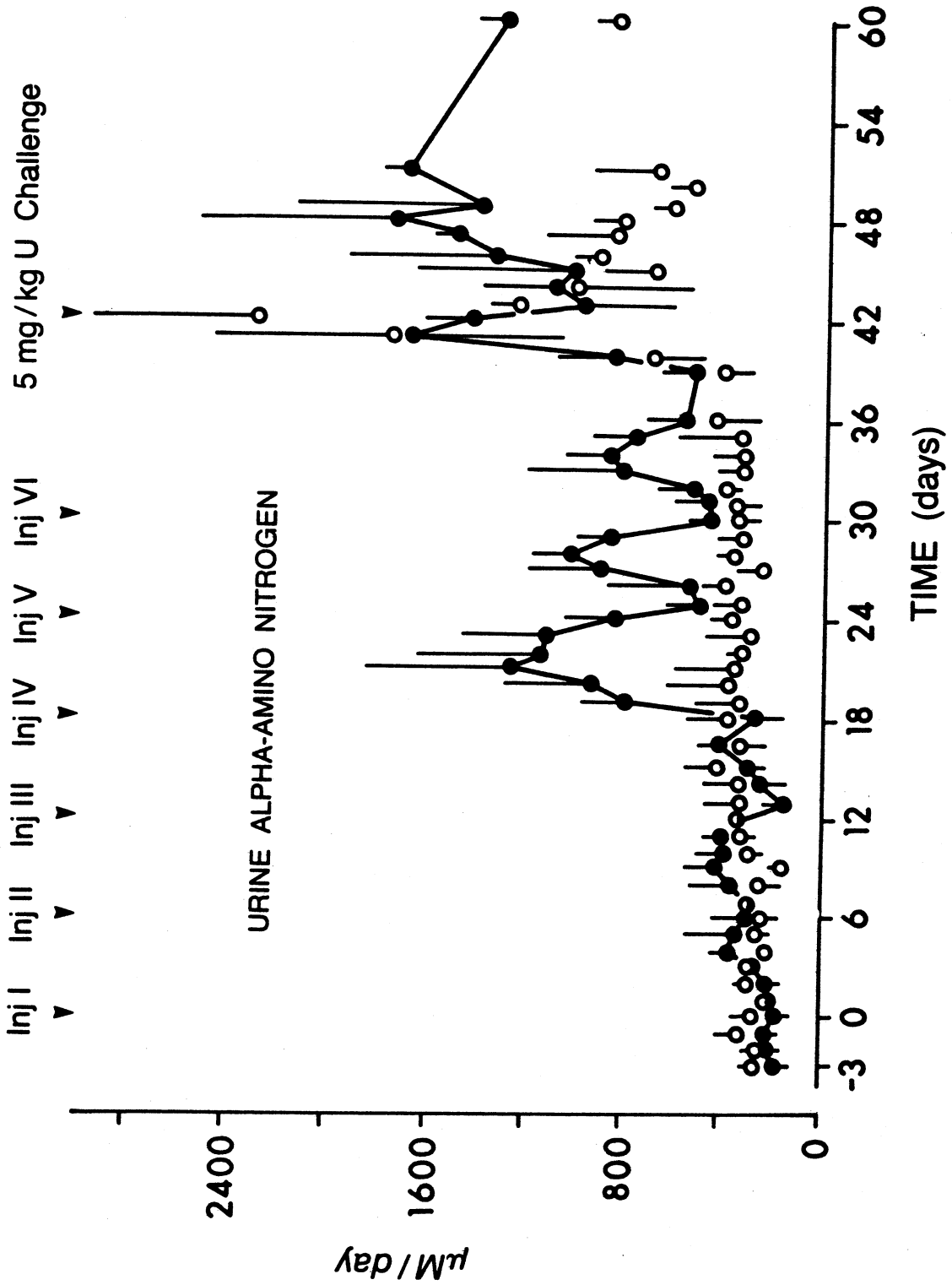

Fig. 31 The effect of multiple low doses of UO_2F_2 (6 x 0.1 mg U/kg) followed by a 5 mg U/kg challenge dose on daily urine amino acid excretion. Solid circles are means ± S.D. of six rats and open circles are means ± S.D. of three saline controls that received the challenge dose.

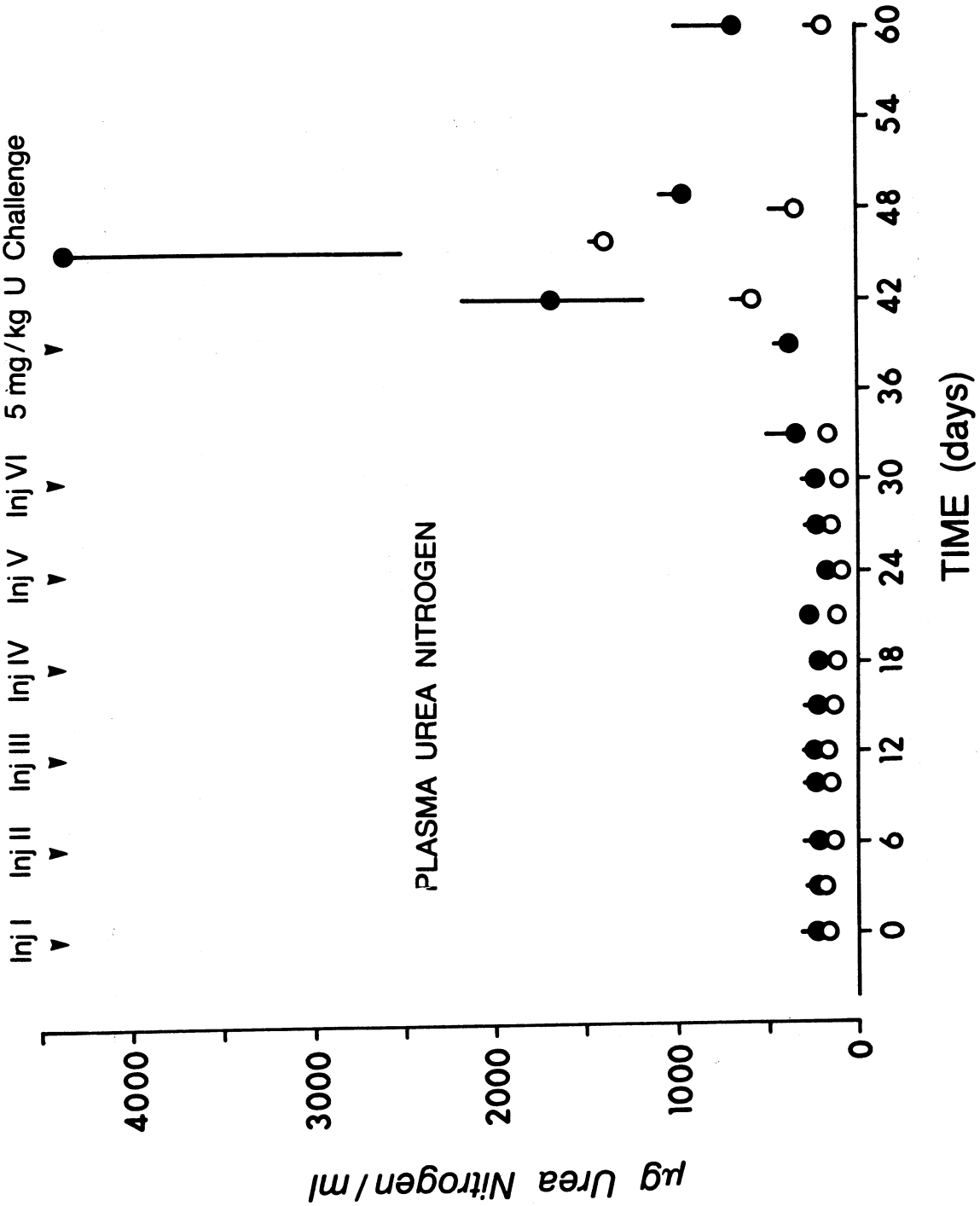

Fig. 32 The effects of multiple low doses of UO_2F_2 (6 x 0.1 mg U/kg) followed by a 5 mg U/kg challenge dose on the plasma urea nitrogen concentration. Solid circles are means ± S.D. of six rats and open circles are means ± S.D. of three saline controls that received the challenge dose.

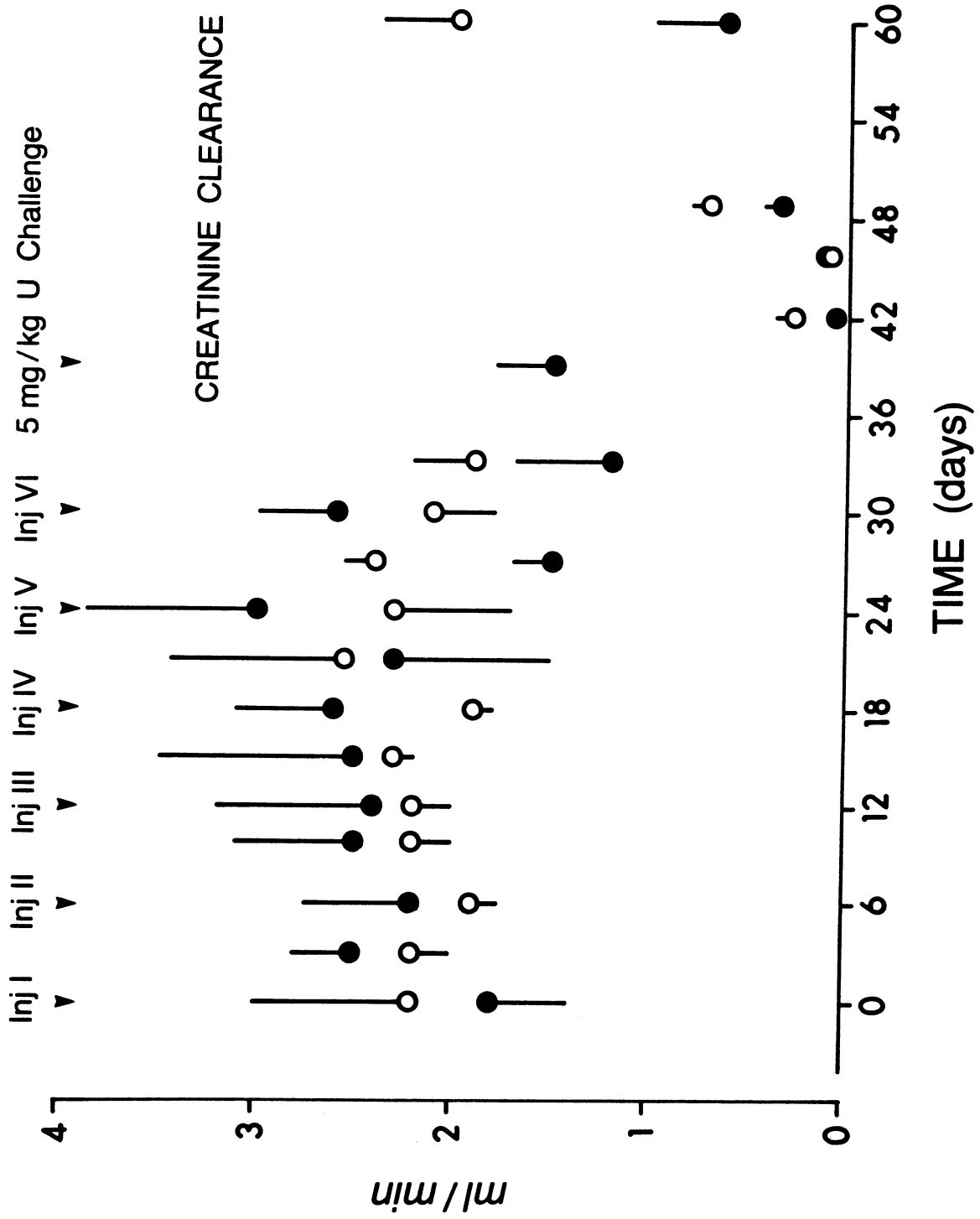

Fig. 33 The effect of multiple low doses of UO_2F_2 (6 x 0.1 mg U/kg) followed by a 5 mg U/kg challenge dose on renal creatinine clearance. Solid circles are means + S.D. of six rats and open circles are means + S.D. of three saline controls that received the challenge dose.

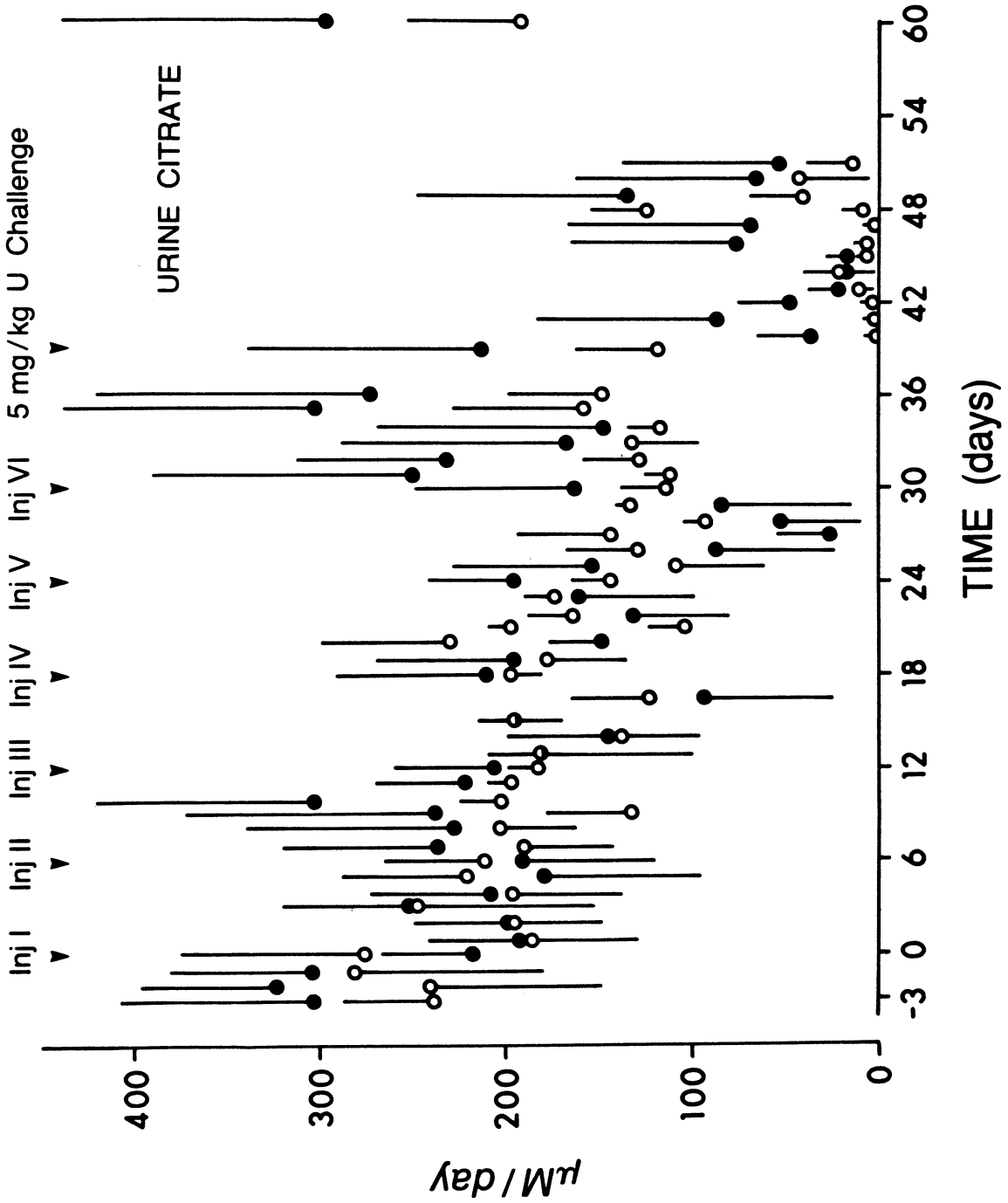

Fig. 34 The effect of multiple low doses of UO_2F_2 (6 x 0.1 mg U/kg) followed by a 5 mg U/kg challenge dose on daily urine citrate excretion. Solid circles are means + S.D. of six rats and the open circles are means + S.D. of three saline controls that received the challenge dose.

higher amounts of UO_2F_2 and found the concentration of uranyl ion did not alter the amount of citrate measured.

Some clear distinctions between the response of naive (untreated) and U^{+6} treated animals to the renal injury indices used in these studies were found when treated and untreated rats received a 5 mg U kg^{-1} test dose (see Figures 26-34). The decline in body weight in naive and U pretreated rats did not differ, as illustrated in Figure 26, however, about a week after the 5 mg U kg^{-1} test dose was given the U pre-treated animals sustained higher water intake and urine output volumes over the following two weeks (Figure 27). Naive rats exhibited the typical proteinuria following the test dose of U^{+6}, but animals given the six 0.1 mg U kg^{-1} "conditioning" doses failed to show proteinuria (values greater than 100 mg day^{-1}) following the 5 mg U kg^{-1} dose (Figure 28). Urine NAG rose dramatically in the naive group reaching a peak daily value of ten to sixteen times normal, however, the urine of the U^{+6} pretreated animals exhibited only a slight increase following the test dose (Figure 29). The glucosuria response to the test dose in the U^{+6} pre-treated rats was attenuated about a factor of ten when compared to naive animal response (Figure 30). Figure 31 illustrates the aminoaciduria that developed after administration of the 5 mg U kg^{-1} test dose. Naive animals showed a peak level of excretion about twice the value obtained from U^{+6} pre-treated animals, however, the "conditioned" rats exhibited a persistent elevation of urine amino acids for at least three weeks, which was approximately six to eight times the initial control values. The 5 mg U kg^{-1} test dose reduced creatinine clearance to almost zero in naive and pre-treated rats. Recovery to normal clearance values was accomplished by the naive animals but the U^{+6} pre-treated animals had a sustained reduction in clearance rates (Figure 33). The plasma urea nitrogen (Figure 32) levels reflect the same point, that is, a persistent decrease in glomerular filtration rate in U^{+6} pre-treated rats for at least three weeks, and probably longer, following the 5 mg U kg^{-1} test dose.

Figures 35 to 42 present time-response data obtained from rats given 1 mg U kg^{-1} by three intravenous injections 14 days apart and then followed by a 5 mg U kg^{-1} test or challenge dose. One animal died 12 days after the first injection; the remaining data are presented as average values for the five survivors. A transient increase in water intake was found following injections one and two, however, following injection three and the 5 mg U kg^{-1} challenge dose, water consumption fell before returning to higher than normal levels. Two and one-half weeks following the test dose, water consumption was about twice that of control values (Figure 35).

Urine output increased to maximum daily volumes about six days following the first two injections of 1 mg U kg^{-1}. This transient response was not seen with subsequent injections. Urine volumes were three to five times normal 18 days after the last 1 mg U kg^{-1} injection and showed greater response to the 5 mg U kg^{-1} dose (Figure 36).

Maximum proteinuria occurred three days after injections one and two, but the proteinuria response following the third injection was greatly reduced. The 5 mg U kg^{-1} challenge dose produced no additional change in the protein concentration in the urine of these experimental animals (Figure 37).

Daily NAG values in the urine showed the usual bimodal excretion pattern after the first 1 mg U kg^{-1} injection but this response became more diffuse with each injection, and the response following the 5 mg U kg^{-1} test dose was greatly reduced (Figure 38).

Although glucosuria was decreased in magnitude with each succeeding 1 mg U kg^{-1} dose, rats still exhibited a 10-fold peak value in glucose excretion which returned to normal in about a week following the 5 mg U kg^{-1} challenge dose (Figure 39).

A marked increase in the excretion of amino acids in the urine was observed after the three 1 mg U kg^{-1} injections, although the response was attenuated following the third dose. A measurable aminoaciduria did occur following the 5 mg U kg^{-1} test dose, but it was much less than the naive rat response (compare Figures 40 and 31).

The clearance of plasma urea nitrogen and creatinine were dramatically reduced after each injection of U^{+6} (Figures 41 and 42). After each dosing, plasma urea nitrogen returned to a stable but elevated condition, indicating continuing damage to the renal tissue that required more than 14 days to repair. Two and one-half weeks following the 5 mg U kg^{-1} challenge, plasma urea nitrogen levels were still five times normal and the creatinine clearance rate remained below 25 percent of control rates. Clearly, these functional tests, as well as increased urine volume, indicate considerable injury to the rat kidney, yet if proteinuria or enzymuria (NAG) and to a lesser extent glucosuria and aminoaciduria were used to evaluate renal function the animals might appear to be more "tolerant" to the effects of U^{+6} than they really are.

Examination of urinary excretion of U following the 1 mg U kg^{-1} injections showed a reduced elimination of the U (approximately 54 percent less) when comparing the first injection with the second and third as illustrated in Figure 43. This figure also shows a diminished U excretion value after the higher 5 mg U kg^{-1} dose of aqueous UO_2F_2.

To further explore the difference in excretion rates when naive and predosed rats are compared, the cumulative urinary excretion patterns for seven days following the 5 mg U kg^{-1} dose in Experiments No. 16 and 18 (Table 6) were plotted in Figure 44. The naive rats (Experiment No. 16) clearly excreted more of the U challenge dose during the first post-treatment week. Thus it would appear that preconditioning the rats with sub-lethal doses of U^{+6} as UO_2F_2 did not increase the rate of urinary excretion of U.

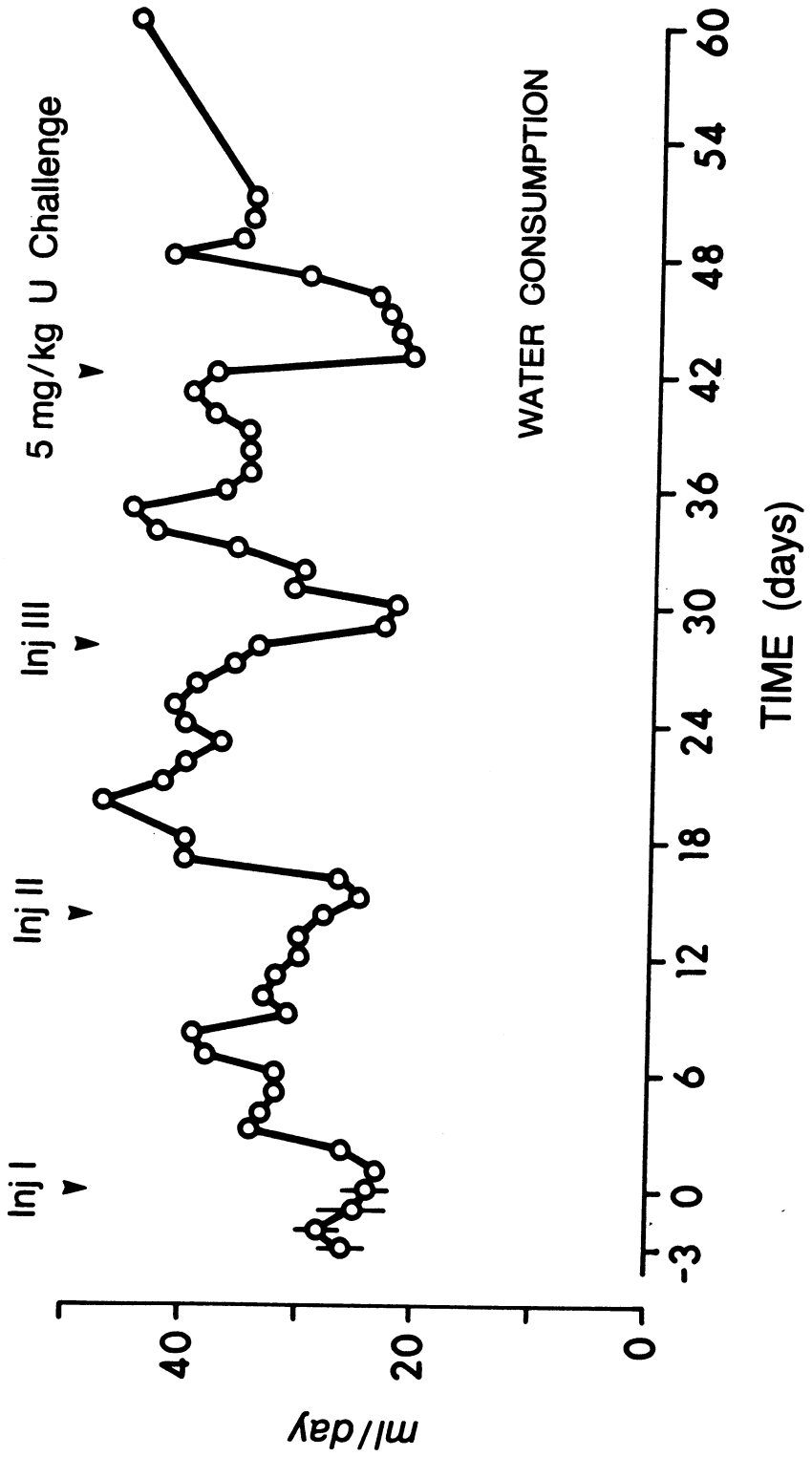

Fig. 35 The effect of three injections of UO_2F_2 (1 mg U/kg) followed by a 5 mg U/kg challenge dose on daily water consumption. Preinjection values are means + S.D. of six rats and subsequent values are averages of at least five animals.

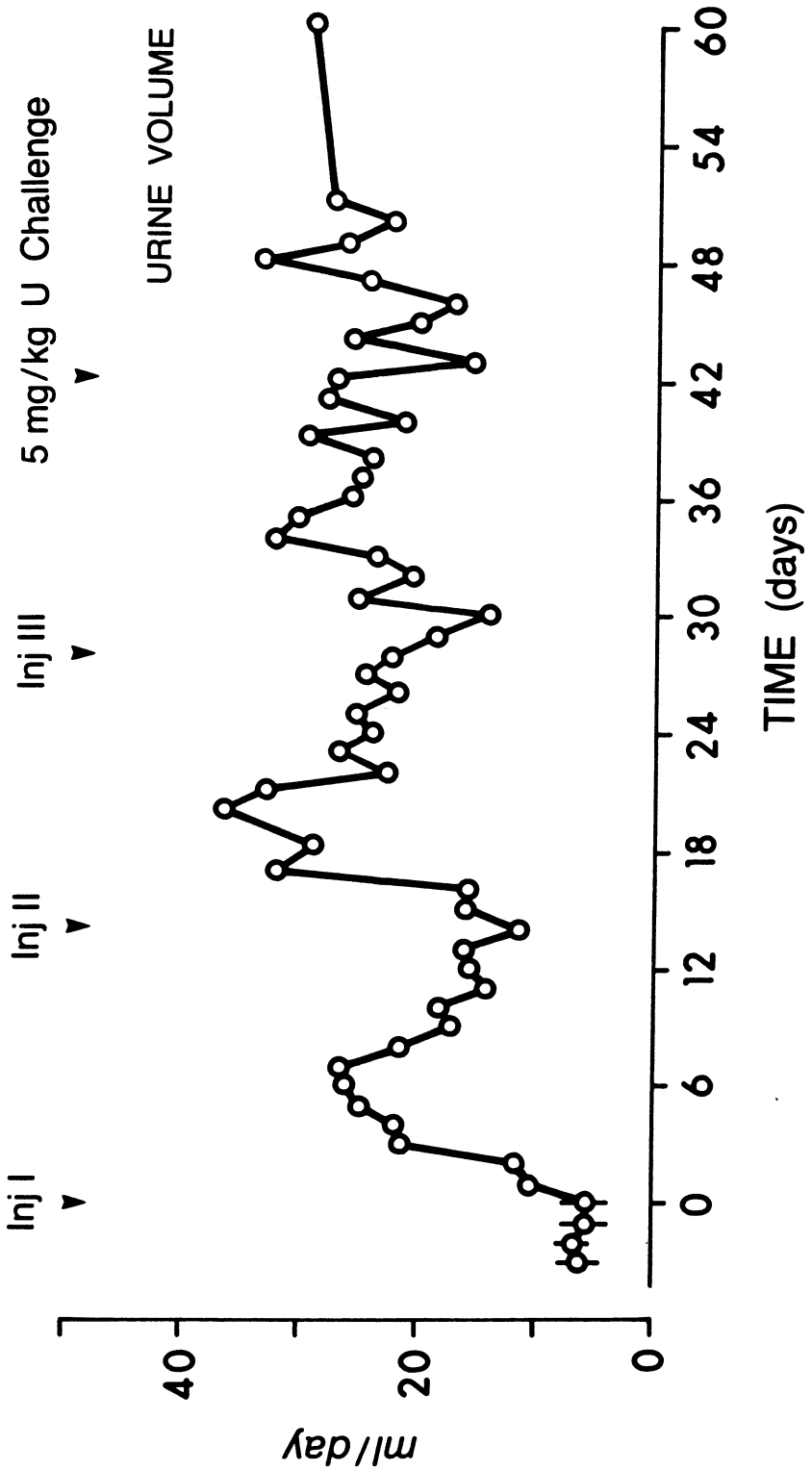

Fig. 36 The effect of three injections of UO_2F_2 (1 mg U/kg) followed by a 5 mg U/kg challenge dose on daily urine volume. Preinjection values are means + S.D. of six rats and subsequent values are averages of at least five animals.

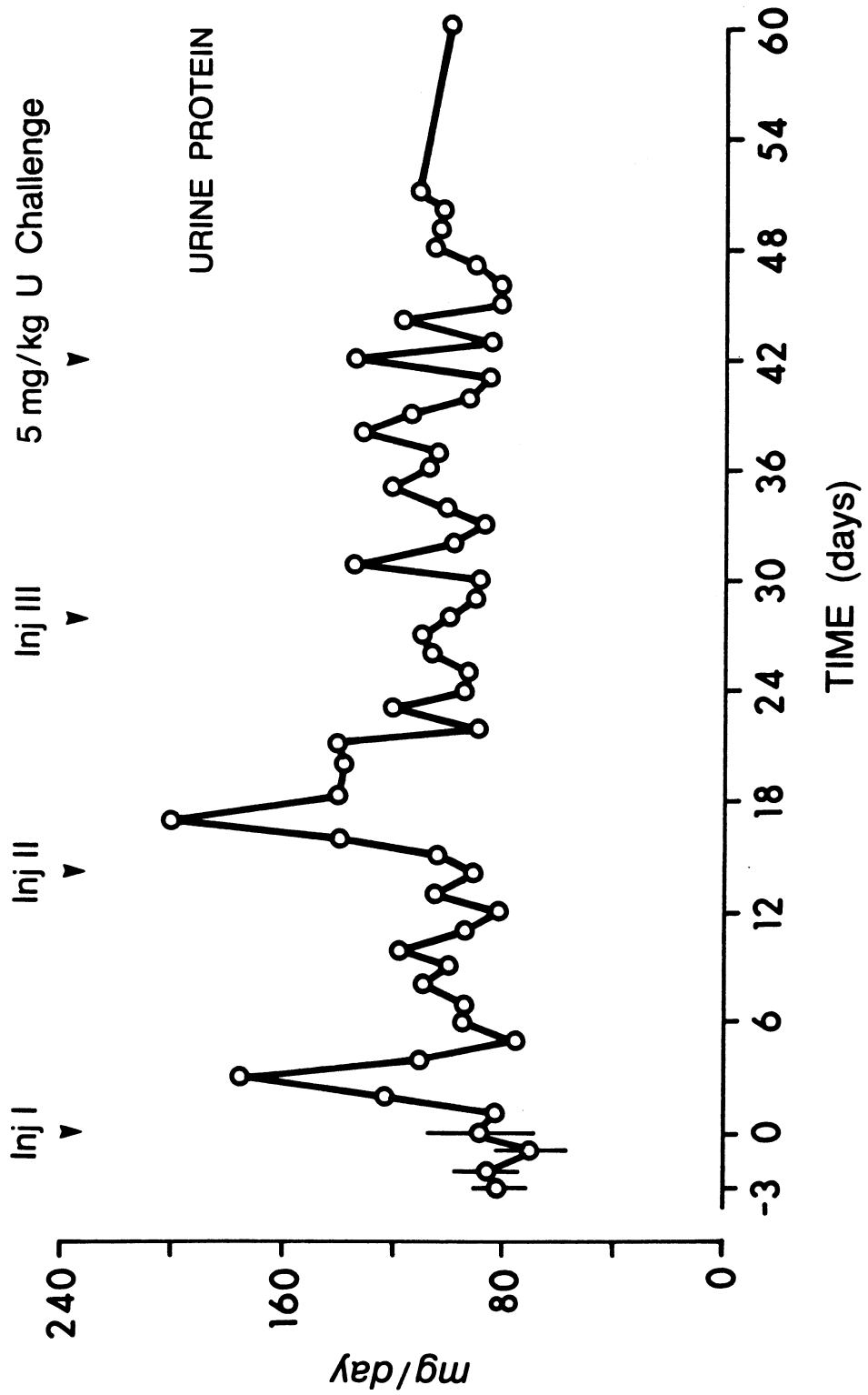

Fig. 37 The effects of three injections of UO_2F_2 (1 mg U/kg) followed by a 5 mg U/kg challenge dose on daily urine protein excretion. Preinjection values are means ± S.D. of six rats and subsequent values are averages of at least five animals.

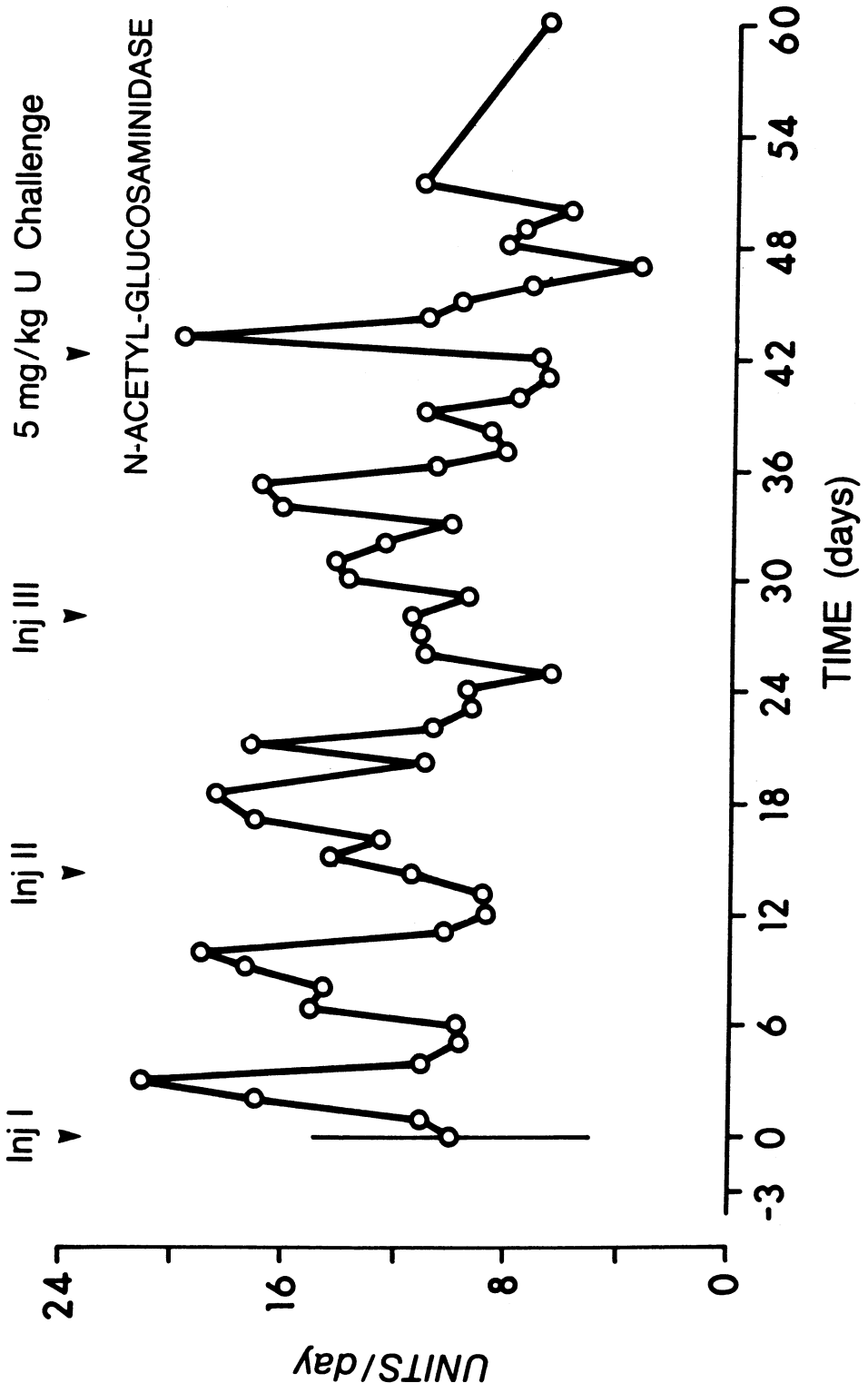

Fig. 38 The effects of three injections of UO_2F_2 (1 mg U/kg) followed by a 5 mg U/kg challenge dose on daily urine N-acetyl-glucosaminidase excretion. Preinjection values are means + S.D. of six rats and subsequent values are averages of at least five animals.

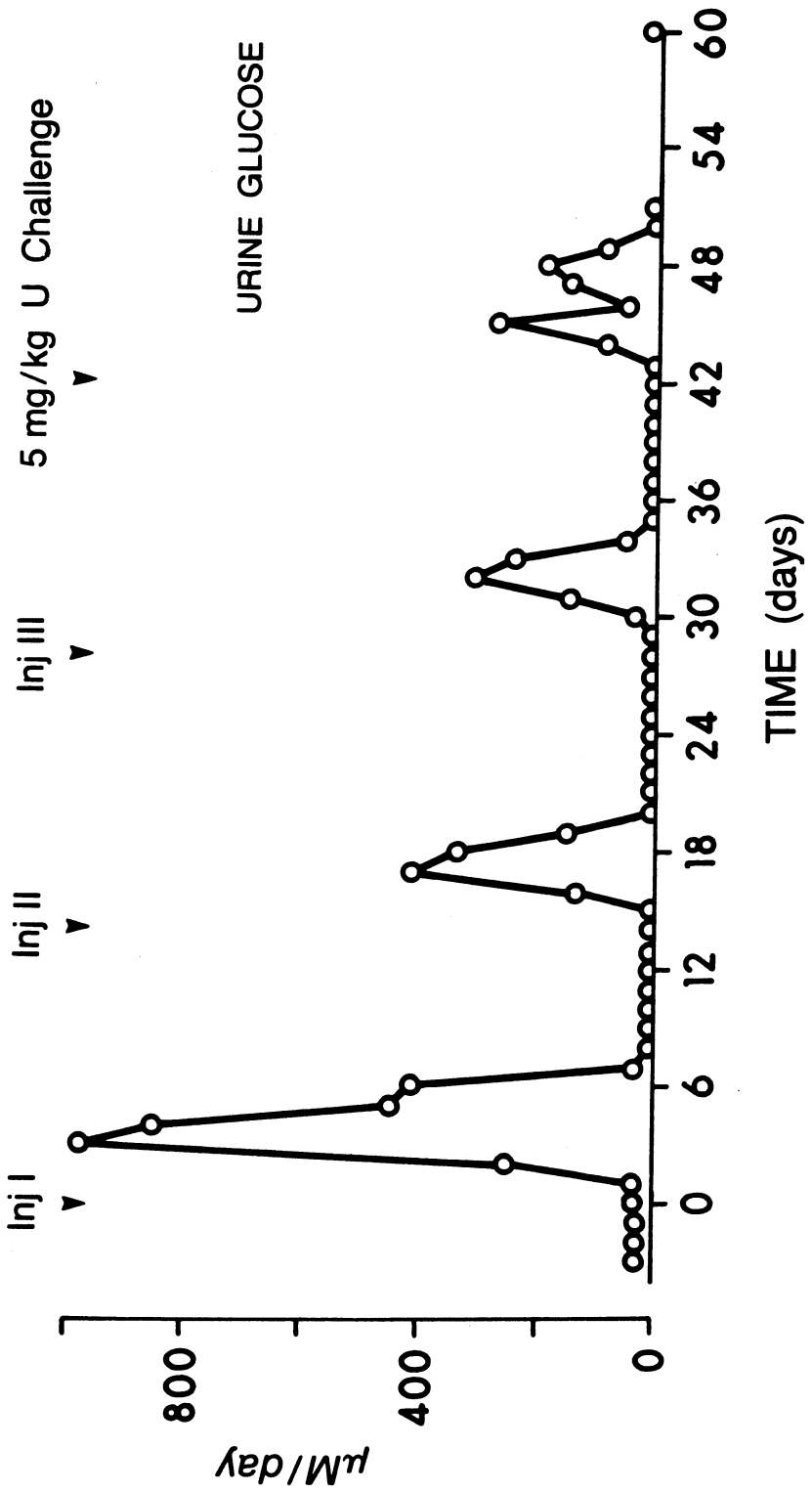

Fig. 39 The effects of three injections of UO_2F_2 (1 mg U/kg) followed by a 5 mg U/kg challenge dose on daily urine glucose excretion. Preinjection values are means of six rats and subsequent values are averages of at least five animals.

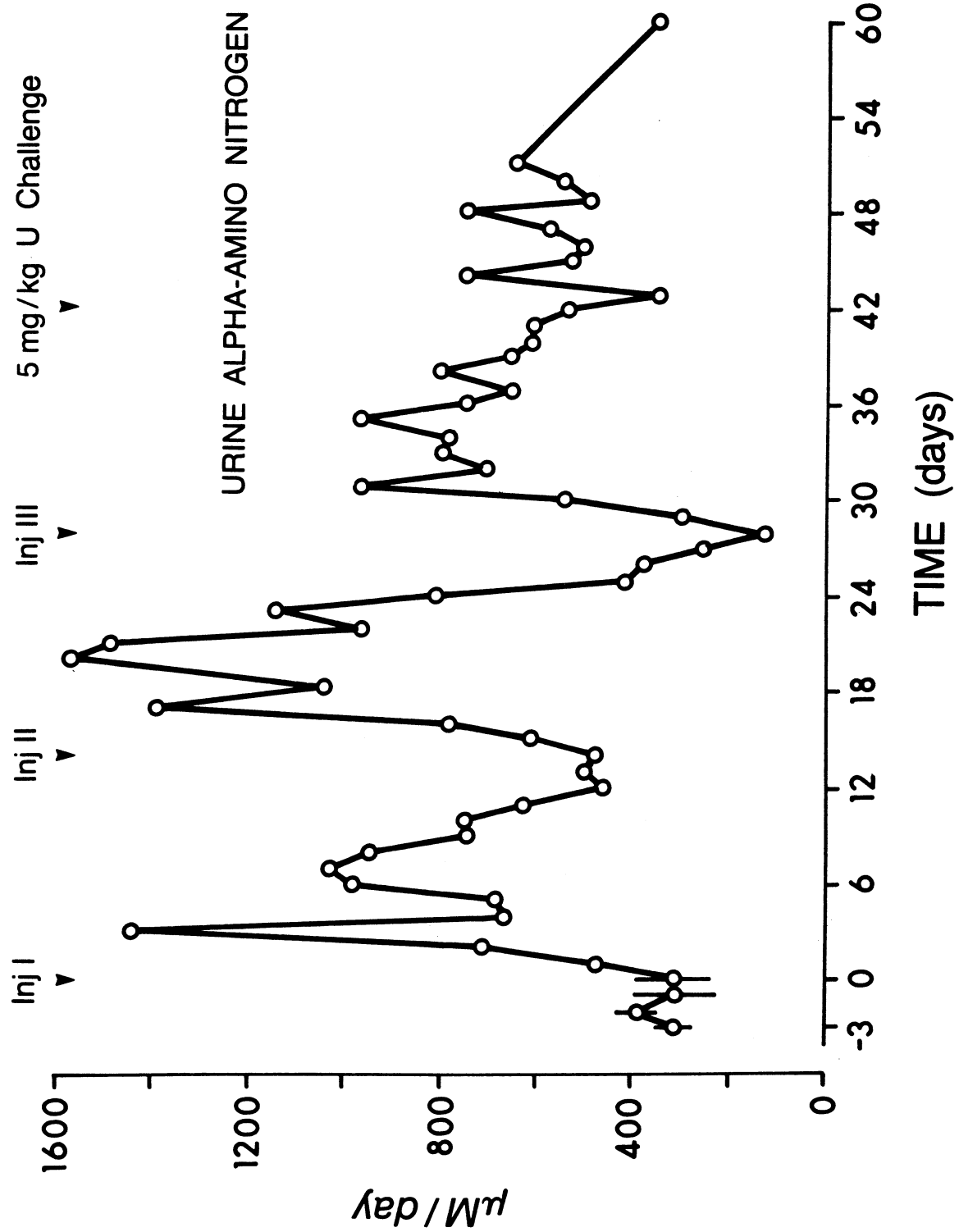

Fig. 40 The effects of three injections of UO_2F_2 (1 mg U/kg) followed by a 5 mg U/kg challenge dose on daily urine alpha-amino nitrogen excretion. Preinjection values are means $\pm$ S.D. of six rats and subsequent values are averages of at least five animals.

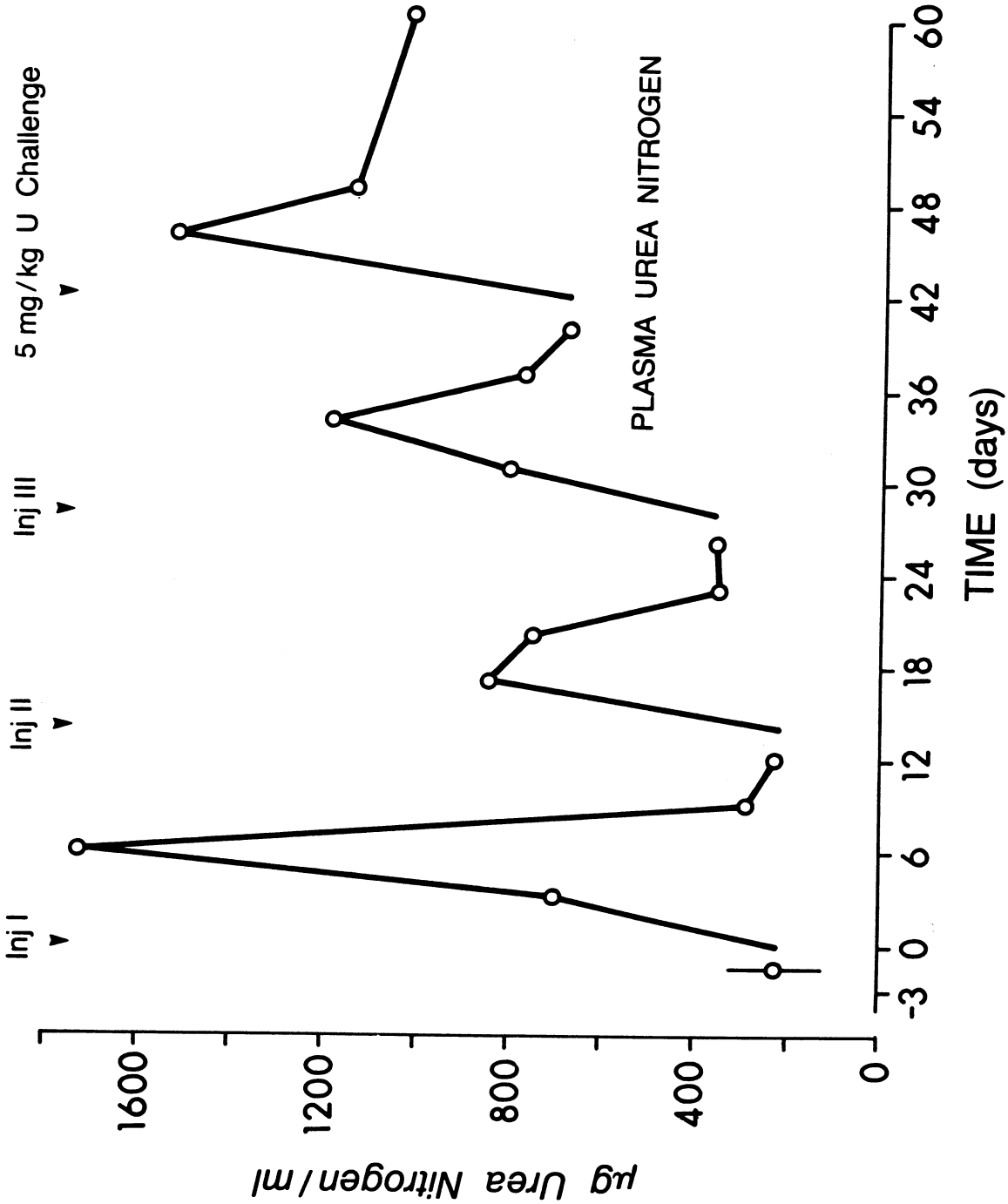

Fig. 41 The effects of three injections of UO_2F_2 (1 mg U/kg) followed by a 5 mg U/kg challenge dose on plasma urea nitrogen concentrations. Preinjection value is mean + S.D. of six rats and subsequent values are averages of at Teast five animals.

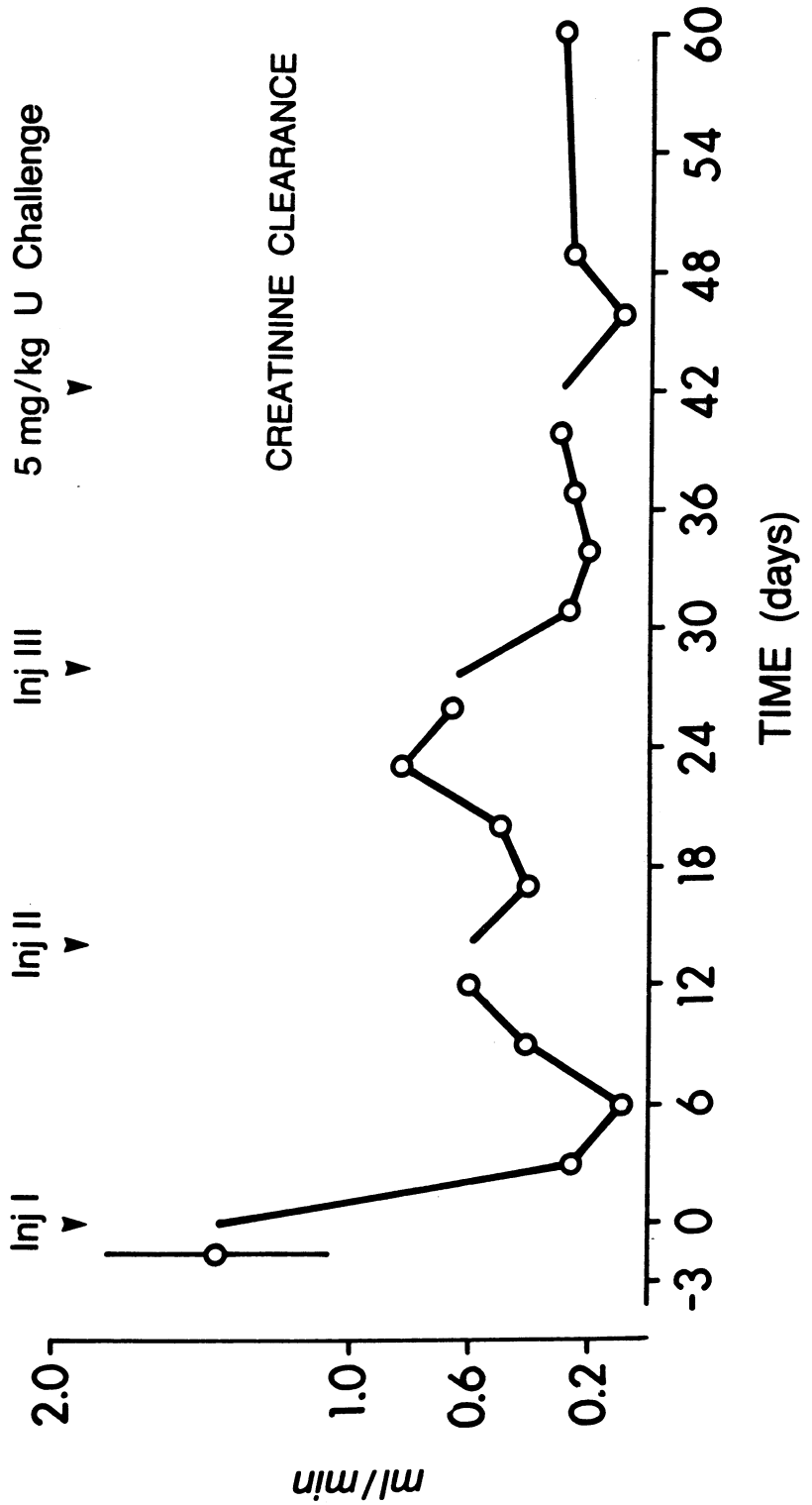

Fig. 42 The effects of three injections of UO_2F_2 (1 mg U/kg) followed by a 5 mg U/kg challenge dose on renal creatinine clearance. Preinjection value is mean ± S.D. of six rats and subsequent values are averages of at least five animals.

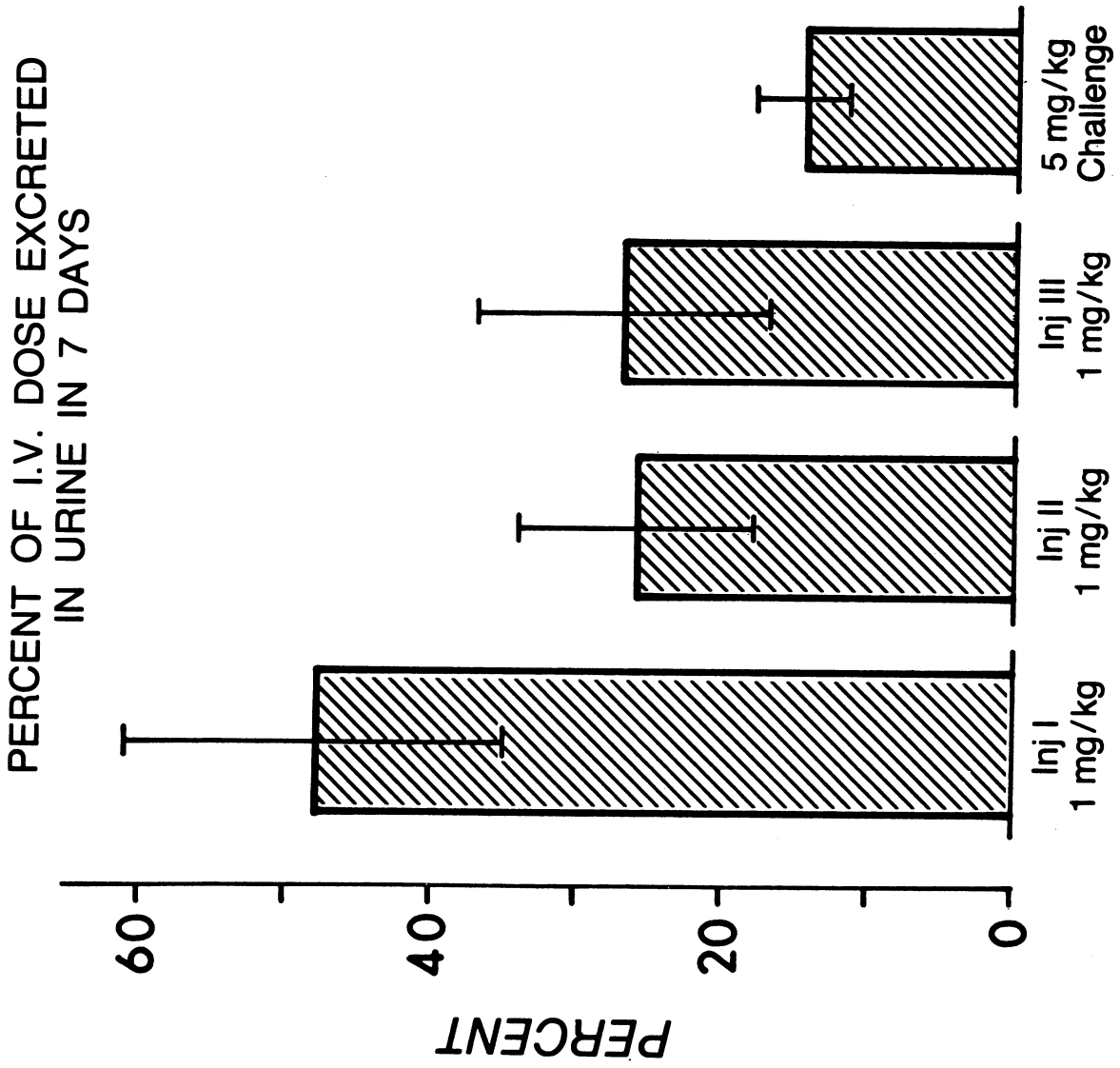

Fig. 43 Uranium excretion: the percent of the injected dose eliminated in the urine during seven day post injection time period. Values are means ± S.D. of at least five animals.

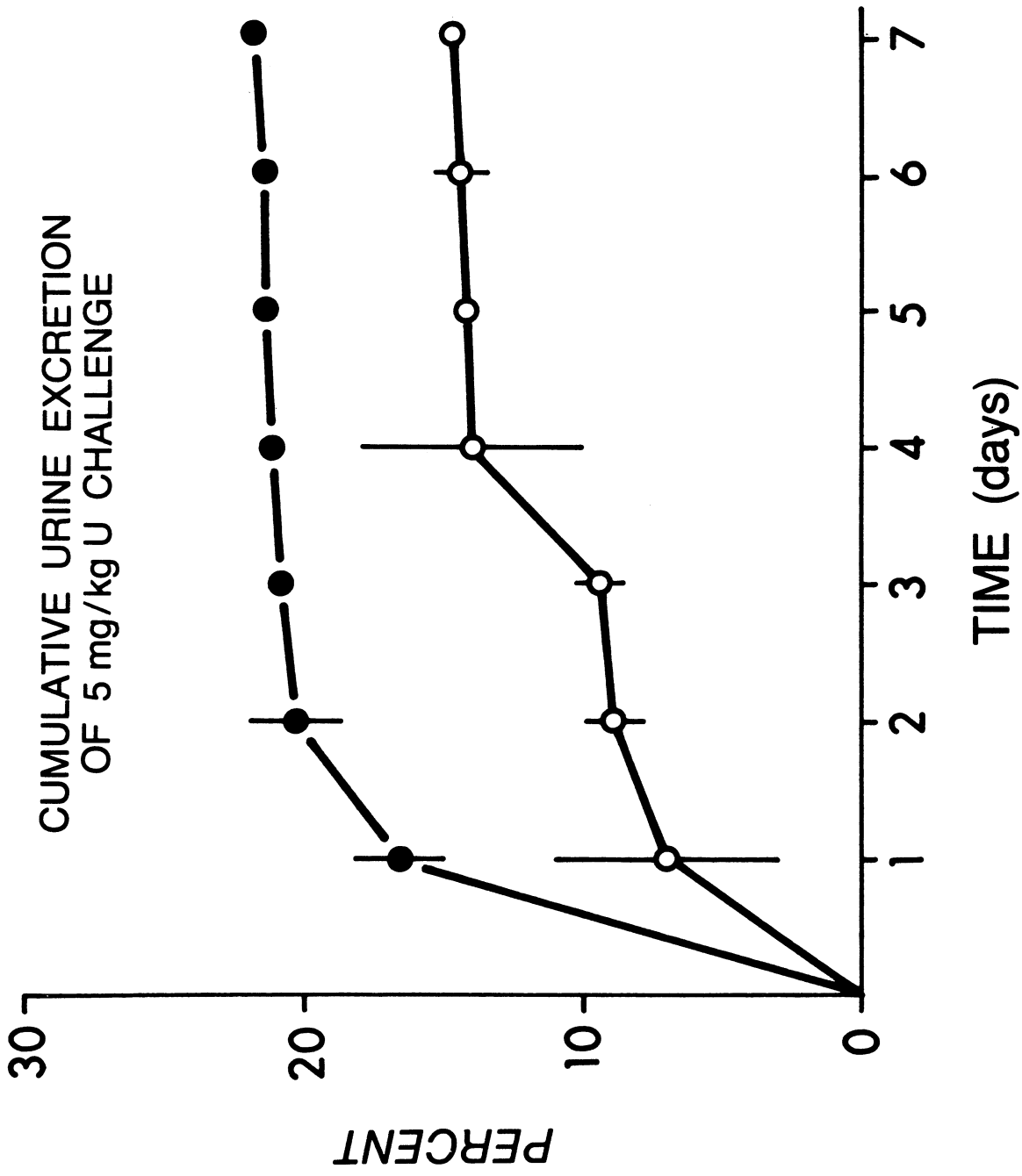

Fig. 44 Comparison between the cumulative uranium urine excretion by naive (solid circles) and preinjected rats (open circles).

F. Toxicity of UO_2F_2 With or Without the Presence of HF

Do the effects of inhalation exposure to UO_2F_2 aerosol in the presence of HF differ from those in a comparable exposure to UO_2F_2 aerosol alone? We have gathered evidence that in the rat under one experimental situation there appears to be a small difference when HF is present. For example, recorded in Table 13 is information obtained from two comparable experiments. Experiment No. 11 (described in detail in this report) was conducted with hydrolyzed UF_6 (UO_2F_2 aerosol + HF gas), while Experiment No. 4 (taken from NUREG/CR-1045, see Table 5) utilized a comparable UO_2F_2 aerosol alone.

Statistical analysis of mean values for urine output, urinary glucose and protein concentrations were different at the 0.05 level of significance with generally higher peak values seen in Experiment No. 11. Only the mean urinary protein values from Experiment No. 11 showed a highly significant (0.01 level) difference when compared with those of Experiment No. 4.

In all other regards, statistical analyses revealed no significant differences in the two experiments. We interpret these data to indicate a slight enhancement of toxicity when UO_2F_2 aerosol is inhaled in the presence of HF by the rat. At higher levels of exposure to hydrolyzed UF_6 (greater than 400 mg U m^{-3} + 70 mg HF m^{-3} for 1.5 hours) HF may exert greater toxicity (See Discussion).

3. Rodent Intratracheal Studies (Smith, Picano, Gelein, Consler, Yuile and Scott)

A. Intratracheal Instillation

Pilot Experiment. To determine an appropriate dosage of UO_2F_2, groups of rats were administered different dosages of UO_2F_2 and the renal burden was determined at intervals thereafter. In two experiments rats were given 2 or 3 mg UO_2F_2 kg^{-1} by intratracheal administration, and the renal burden of uranium was determined 21 and 28 days later. In two other experiments rats received 0.1 or 1.0 mg UO_2F_2 kg^{-1} and the mean renal burden was determined at intervals out to 21 days post-instillation. Histological findings in the lungs and kidneys of these rats have been described earlier (Morrow et al., 1980).

On the basis of these data it was decided to use a dosage of 2.0 mg UO_2F_2 kg^{-1} to examine more carefully the effects of this dosage given initially and then again 60 days later. This dosage would be expected to produce more than marginal, but not intolerable, renal damage clearly evident by biochemical and histologic changes.

Effects of Two Successive Doses of 2 mg UO_2F_2 kg^{-1} Body Weight. For this experiment 70 male Sprague-Dawley rats initially weighing 200-220 g were divided into groups of 35 animals each,

Table 13. A comparison of selected toxicologic data from Experiment No. 11 and Experiment No. 4 NUREG/CR-1045, see Table 5).

Experiment No.	Test material	Aerosol conc., mg U M^{-3}	Duration of exposure, hr	Particle size MMAD, μm	σ_g
11	UO_2F_2 + HF	357 ± 89	1.5	1.59	1.92
4	UO_2F_2	234 ± 15	1	1.40	1.94

	Mortality figures No. dead/No. used	Mean inhaled dose mg U kg^{-1}	Mean absorbed dose mg U kg^{-1}
11	2/11	7.6	2.5
4	1/6	6.1	2.4

Mean percent of absorbed dose at 14 or 15 days postexposure

	Urine	Bone	Kidneys	Lungs
11	53	37	5	5
4	68	23	5	3

Mean percent of inhaled dose at 10 days postexposure

	Feces	Urine	Total excretion
11	65	17	82
4	59	23	82

	Number	Species	Strain	Sex
11	11	Rat	LE	Male
4	6	Rat	SD	Male

identified as Group I and II. Eight animals of Group I and 10 of Group II were designated as controls. All of the remaining animals received a single intratracheal instillation of 2 mg UO_2F_2 kg^{-1} body weight; those of Group II received the UO_2F_2 3 days after those of group I. Solutions of UO_2F_2 proved to be highly acidic; therefore, the compound was administered in Tris buffer, pH 6.90, in a volume of 0.5 ml kg^{-1} body weight. The control rats received an intratracheal instillation of Tris buffer only, 0.5 ml kg^{-1} of body weight. The animals of Group I were housed individually in metabolism cages, while those of Group II were placed in ordinary living cages. During the 60 days following instillation, for animals of Group I food and water consumption and urine excretion were measured daily during the first 7 days, and at intervals thereafter. Feces of the dosed animals were collected daily during the first four days. During days 3-6, blood was obtained from groups of 3 control animals and from 1-4 dosed rats for plasma urea N. Beginning on day 7, post-instillation groups consisting of one control rat and 2 or 4 dosed rats were sacrificed at intervals. Blood was removed for urea N measurement, the kidneys were removed for histological examination and for determination of uranium content, and sections of the lung and trachea were taken for histological examination.

On day 58 post-instillation the animals of Group II were moved to metabolism cages and after 3 days of acclimatization, i.e. on day 61 post-instillation, the dosed animals were given a second intratracheal instillation of 2 mg UO_2F_2 kg^{-1} body weight. Food and water intake was measured, urine and fecal collections were made, and serial sacrifices done for these Group II rats during the second 60 days on the same schedule followed for Group I rats during the initial 60 days. Blood samples for urea N, kidneys for uranium analysis, and kidney, lung and trachea sections for histological examination also were taken on the same schedule as before.

All assignments of animals to Group I or Group II, to control and dosed groups, and to sacrifice groups were made on a random number selection basis.

During the first 7 days post-instillation all surviving dosed and control animals were monitored daily for most parameters and the means and standard deviations could be calculated and plotted. Thereafter, the number of control and dosed animals progressively decreased to 1 and 4 for Group I and to 2 and 4 animals for Group II. The weighting for each datum plotted therefore progressively decreased through day 14-60. Accordingly, for this latter time interval only mean values were plotted and considered as representing trends.

B. Uranium Excretion

Figure 45A and B shows the excretion of uranium by once and by twice dosed animals during the first 7 days after administration of UO_2F_2. A striking difference is evident between the two

groups. Following a single dose of 2 mg UO_2F_2, excretion declined slowly, from a maximum of 2.2 μg U rat^{-1} day^{-1} at day 1 to a level of 1.65 μg U rat^{-1} day^{-1} at day 5. Thereafter, the excretion dropped sharply to 0.19 and 0.10 μg U for days 6 and 7. In the twice dosed rats however, excretion on the first day after the second dose was approximately 100 μg U/rat/day. Thereafter, excretion dropped precipitously to 4.3 μg on day 5 and then to undetectable amounts on day 7.

Inasmuch as excretion of uranium was not measured in the pilot experiments and because the dosage of uranium was constant in the two-dose experiment, a dose-response relationship for uranium excretion cannot be developed.

In the two-dose experiment urinary excretion of uranium after the first dose declined slowly during the first 5 days post-instillation, and then dropped abruptly during the next two days. The cumulative excretion of uranium during the first 7 days was approximately 10 μg (Figure 45A). Assuming urinary excretion to be negligible thereafter, the data show that excretion of uranium in the urine in the first 24 hours following the initial dose was approximately 22 percent of the total urinary excretion for the first week post administration. The twice dosed rats excreted approximately 135 μg of uranium via the kidneys during the first 7 days. About 76 percent of this total appeared in the first 24 hours. Given the extent and rapidity with which UO_2F_2 is known to be absorbed from the lungs, it can be assumed that the amount of UO_2F_2 given by intratracheal instillation closely approximates the absorbed dose. The fraction, then, of the first absorbed dose (355 μg U administered per rat) recovered in the 7 days post instillation is 2.8 percent and the fraction excreted in the first 24 hours is approximately 0.6 percent. Corresponding fractions after the second administration (391 μg U per rat) are 34.5 percent and 26 percent. These percentages are appreciably less than Morrow et al. found in the dog. The kinetics of the urinary excretion of uranium in the rats dosed again 60 days after the initial dosing thus appear to be quite different from that in once dosed rats, in that the twiced dosed animals excreted a greater fraction of the absorbed dose in the first week, and a greater fraction of that excreted appeared in the first day. Excretion was more rapid in the twice dosed rats. The increased excretion of uranium by the rats receiving two doses of UO_2F_2 60 days apart may reflect the greater histological damage produced in the twice dosed animals, and may also be attributed, at least in part, to the greater volume of urine excreted by these animals.

It should be pointed out that the course of urinary excretion of uranium during the first week following the initial instillation of 2 mg UO_2F_2 kg^{-1} did not follow the pattern seen by Leach and Gelein (Morrow et al., 1980) in the rat after a single intratracheal instillation of approximately 8 mg UO_2F_2 kg^{-1}. These latter authors found approximately 30 percent of the absorbed dose in the urine after one day. Total excretion during the first week was

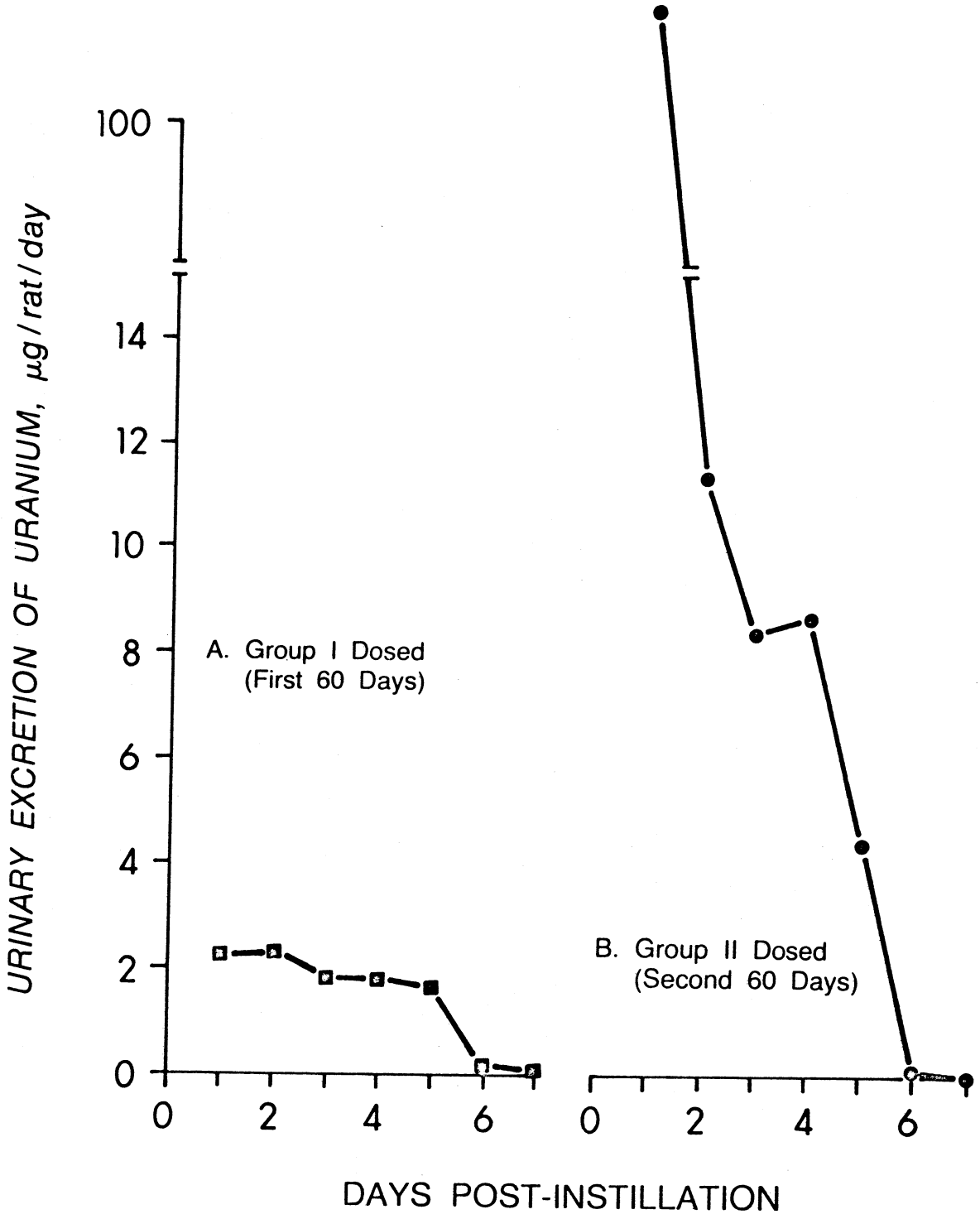

Fig. 45 Daily urinary excretion of uranium after one (□) and after two (○) instillations of 2 mg UO_2F_2/kg.

about 36 percent of the dose. A prompt outpouring of uranium in the urine was seen however, following the second intratracheally-administered dose of UO_2F_2, similar to that after one exposure to uranium (See VI.2.B).

Fecal excretion was measured only during the first four days post-instillation, inasmuch as earlier experience indicated that the bulk of fecal excretion occurred during this early period. The daily excretion per rat for once- and twice-dosed rats is shown in Figure 46A and 46B. These limited data indicate that generally daily fecal excretion was initially high and declined rapidly. Figure 46B indicates that the twice-dosed animals excreted more uranium than did the once-dosed rats. This is confirmed by comparing the fractions of the administered doses recovered in the feces during the first 4 days post-instillation. The average amount of uranium given the once dosed animals was 355 μg U rat^{-1}; the total amount recovered in the feces during the first 4 days was 5.2 μg rat^{-1}, or 1.5 percent of the dose. The second dose given to the twice dosed rats was 391 μg U rat^{-1} and the total uranium excreted in the feces during the ensuing 4 days was 11.8 μg U rat^{-1}, corresponding to 3.0 percent of that administered. In both instances the fecal uranium presumably represents material moved up the tracheobronchial tree and swallowed, as well as material which may have been delivered to the intestinal tract via the bile.

C. Uranium Retention

The earliest samples obtained for determination of renal burdens of uranium were taken at 3 days post administration, in the pilot experiments, and at 7 days post administration in the two dose experiment. Consequently, no data are available to show a build-up of uranium in this organ in the hours immediately after administration.

Data describing the renal retention of uranium after dosages of 0.1 or 1.0 mg UO_2F_2 kg^{-1} (Table 14) are plotted in Figure 47. In both situations removal of uranium from the kidneys during the first 21 days post-instillation can be described by first order kinetics. Even though there was a 10-fold difference in the amounts of uranium administered, the half-times were nearly identical, being 5.3 and 5.1 days for rats receiving 0.1 and 1.0 mg UO_2F_2 kg^{-1}, respectively. The initial renal concentration of uranium was about 15 times greater in those rats receiving the higher dose.

Following a dosage of 2.0 mg UO_2F_2 kg^{-1} body weight, the mean renal burdens of U at 21 and 28 days were 7.3 $\pm$ 5.3 and 4.9 $\pm$ 1.1 μg g^{-1} tissue, respectively. At 21 and 28 days after receiving 3.0 mg UO_2F_2 kg^{-1}, the renal burdens were 13.4 $\pm$ 10.7 and 9.6 $\pm$ 4.2, respectively.

In the two dose experiment utilizing 2 mg UO_2F_2 kg^{-1}, where information was available out to 60 days after each dose, the renal burden also declined with time (Figure 48). Following the first

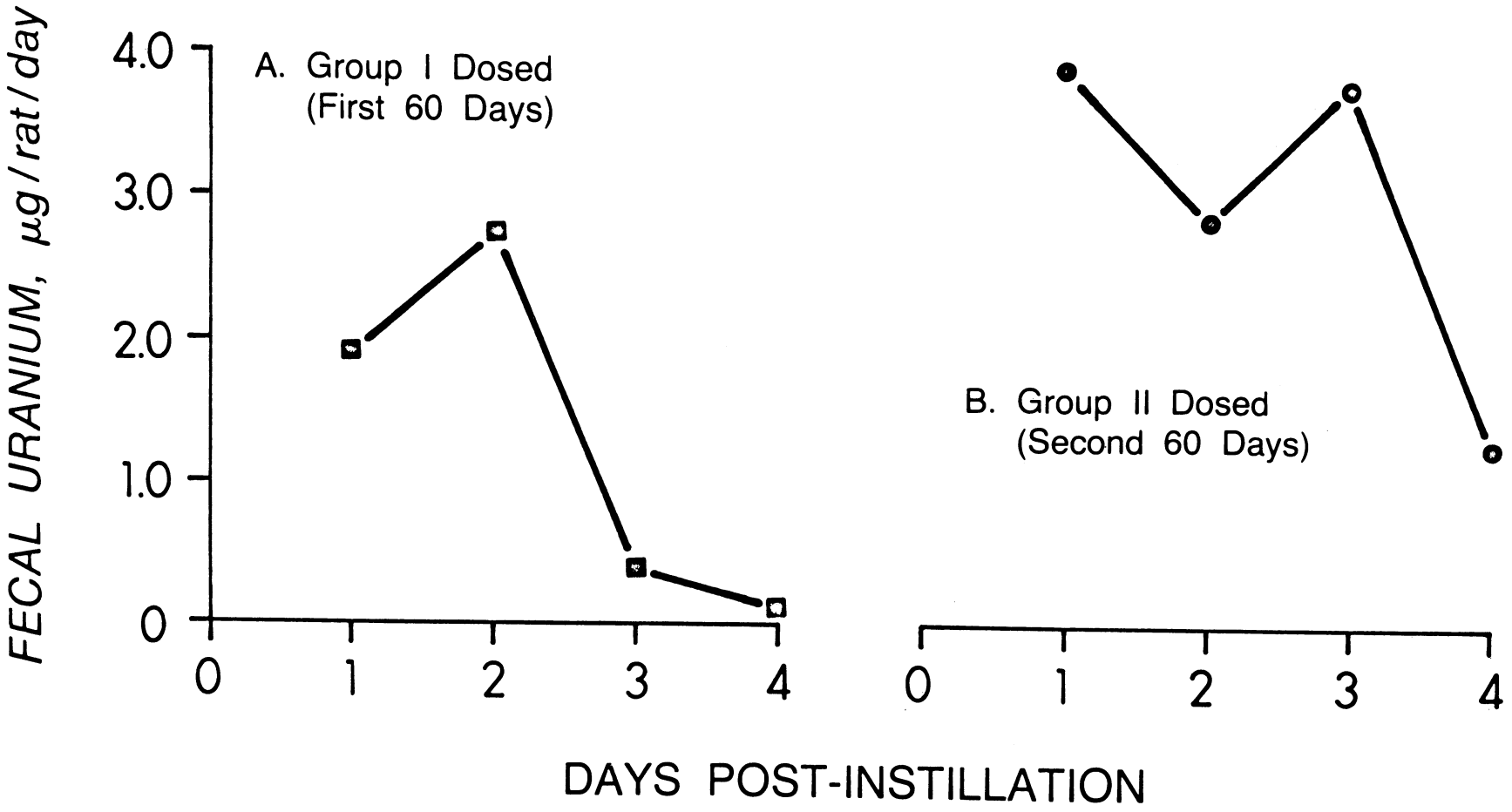

Fig. 46 Daily fecal excretion of uranium after one (◘) and after two (⊙) instillations of 2 mg UO_2F_2/kg.

Table 14. Renal burden of uranium at intervals after intratracheal instillation of 0.1-3 mg UO_2F_2 kg^{-1}

Days post-instillation	mg UO_2F_2/kg body weight			
	0.1*	1.0*	2.0	3.0
	μg U g^{-1} kidney, mean ± S.D.			
3	1.55 ± 0.99	21.4 ± 4.44		
7	0.42 ± 0.36	13.9 ± 2.43		
10	0.76 ± 0.09	9.1 ± 1.79		
14	0.70 ± 0.22	4.92 ± 2.49		
17	0.29 ± 0.15	2.25 ± 0.44		
21	0.08 ± 0.07	2.43 ± 0.21**	7.3 ± 5.3†	13.4 ± 10.7
28	-	4.9 ± 1.1[c]	9.6 ± 4.2	

* n = 3 except as noted

** n = 2

† n = 4

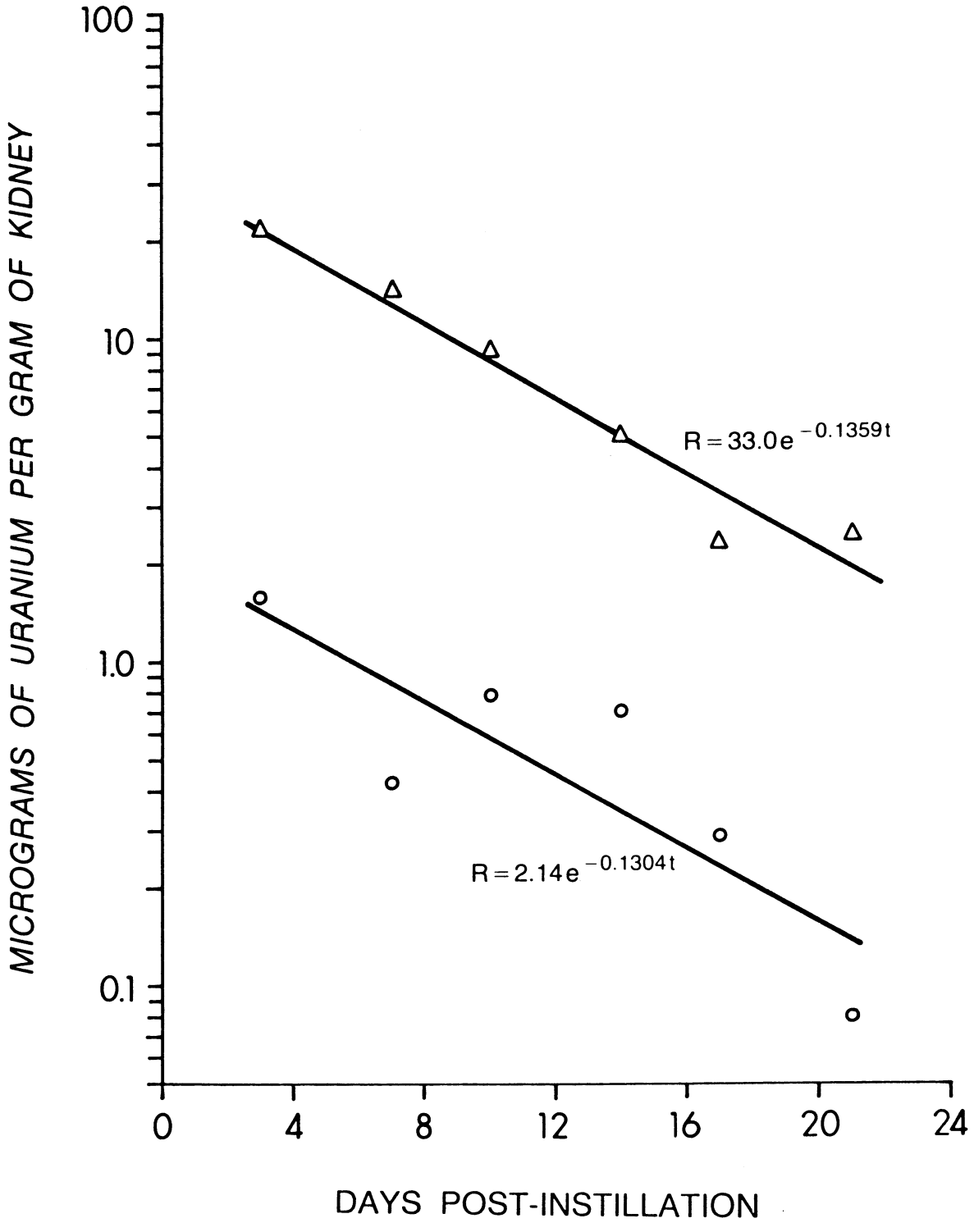

Fig. 47 Renal retention of uranium after dosages of 0.1 (o) or 1.0 (Δ) mg UO_2F_2/kg.

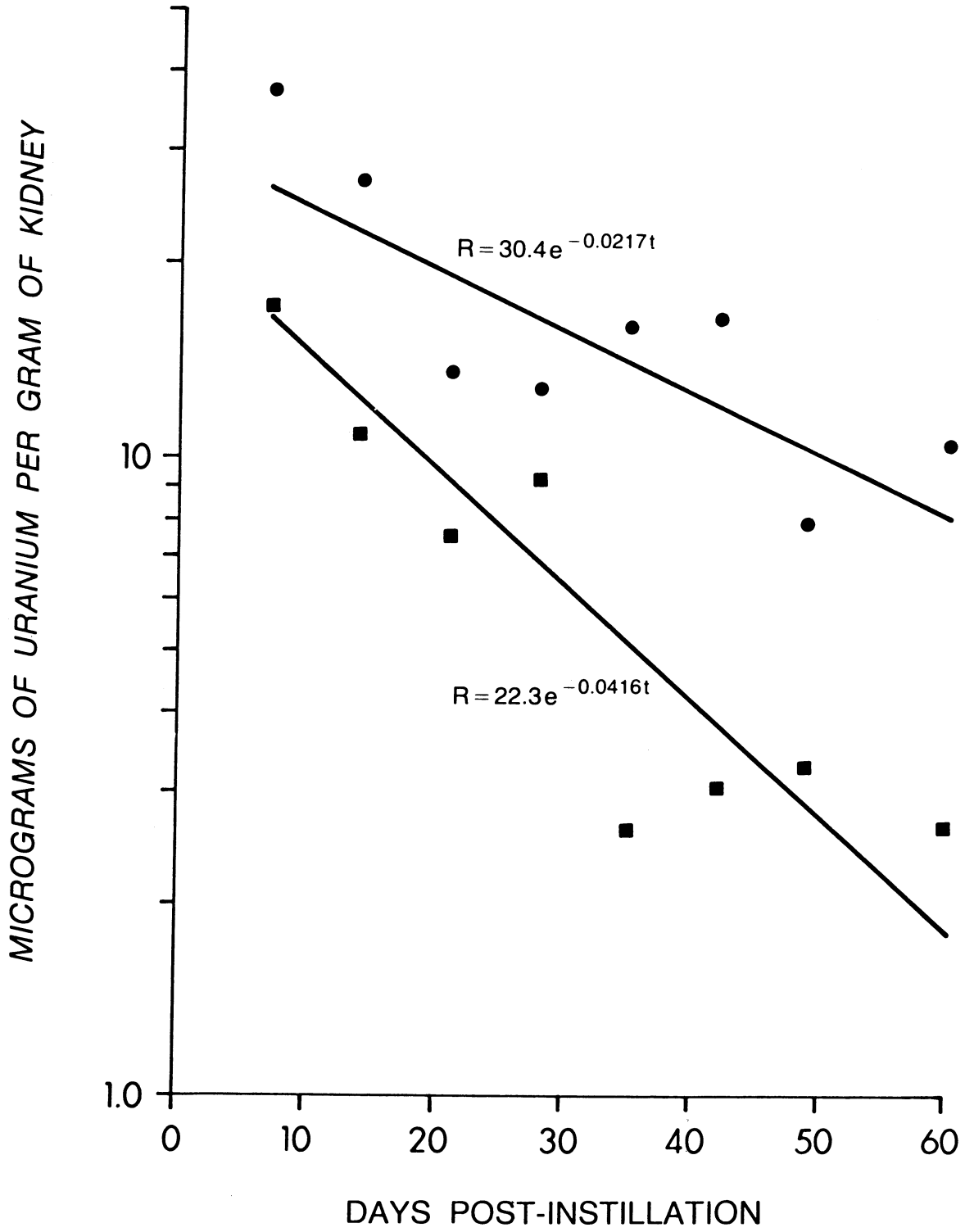

Fig. 48 Renal retention of uranium after one (■) and after two (●) instillations of 2 mg UO_2F_2/kg.

dose of 2 mg UO_2F_2 kg^{-1} the decrease in renal burden of uranium was characterized by a half-time of 16.7 days. This is about 3-fold greater than the half-time observed following administration of 1.0 mg UO_2F_2 kg^{-1} and probably reflects chiefly the greater duration of the experiment. Following the second dose of UO_2F_2 in the two-dose experiment, the half-time for removal of uranium from the kidney was 32 days. In addition, the initial renal burden after the second dose was approximately 36 percent greater than after the first dose. The evidence suggests that the kidneys of the twice dosed rats were less efficient in removing uranium than were those of animals receiving only the one dose.

Lung concentrations were highest (7-8 μg U g^{-1}) during the first three weeks after instillation and declined slowly thereafter (Figure 49A and B). In both groups of rats there is some evidence that a portion of that uranium reaching the lungs after intratracheal instillation was removed at a slow rate.

Figure 49C and D shows that little uranium remained in the trachea at the site of injection. Figure 49D indicates that the twice-dosed animals retained slightly more uranium than did the once-dosed rats, but the difference is not appreciable. However, both groups did retain small amounts of uranium at the injection site at least for as long as 60 days after instillation.

D. Urine Biochemistry

a) Protein: The effect of intratracheally-instilled UO_2F_2 on the urinary excretion of protein is shown in Figures 50-53. During the first week post-instillation control animals of the two groups excreted 50-100 mg of protein daily (Figure 50A and B). Excretion appeared to be somewhat greater in the Group II controls (Figure 50B), but considerable variability from day to day is evident for both groups and the difference is not considered meaningful. Following the first administration of UO_2F_2 urinary protein increased each day to a maximal level on day 4 post-instillation (Figure 50C). Near normal levels were reached again on day 7. Considerable overlap from day to day is evident, however. In rats receiving a second instillation of UO_2F_2 protein excretion again increased promptly (Figure 50D). Maximal excretion appeared one day earlier than after the first instillation, but in view of the great variability from day to day in both groups no significance should be attached to this observation. Figures 52A and B, and Figure 52C and D demonstrate again that in the first week following administration of UO_2F_2 urinary protein excretion is sharply elevated, with some rats showing extremely high amounts. Figure 53A and B, and 53C and D show that in rats dosed one or two times urinary protein excretion had returned to normal after the first week post-instillation. Figure 51C and D suggest the twice dosed rats excreted slightly more protein than did the once dosed animals, but the difference is not striking.

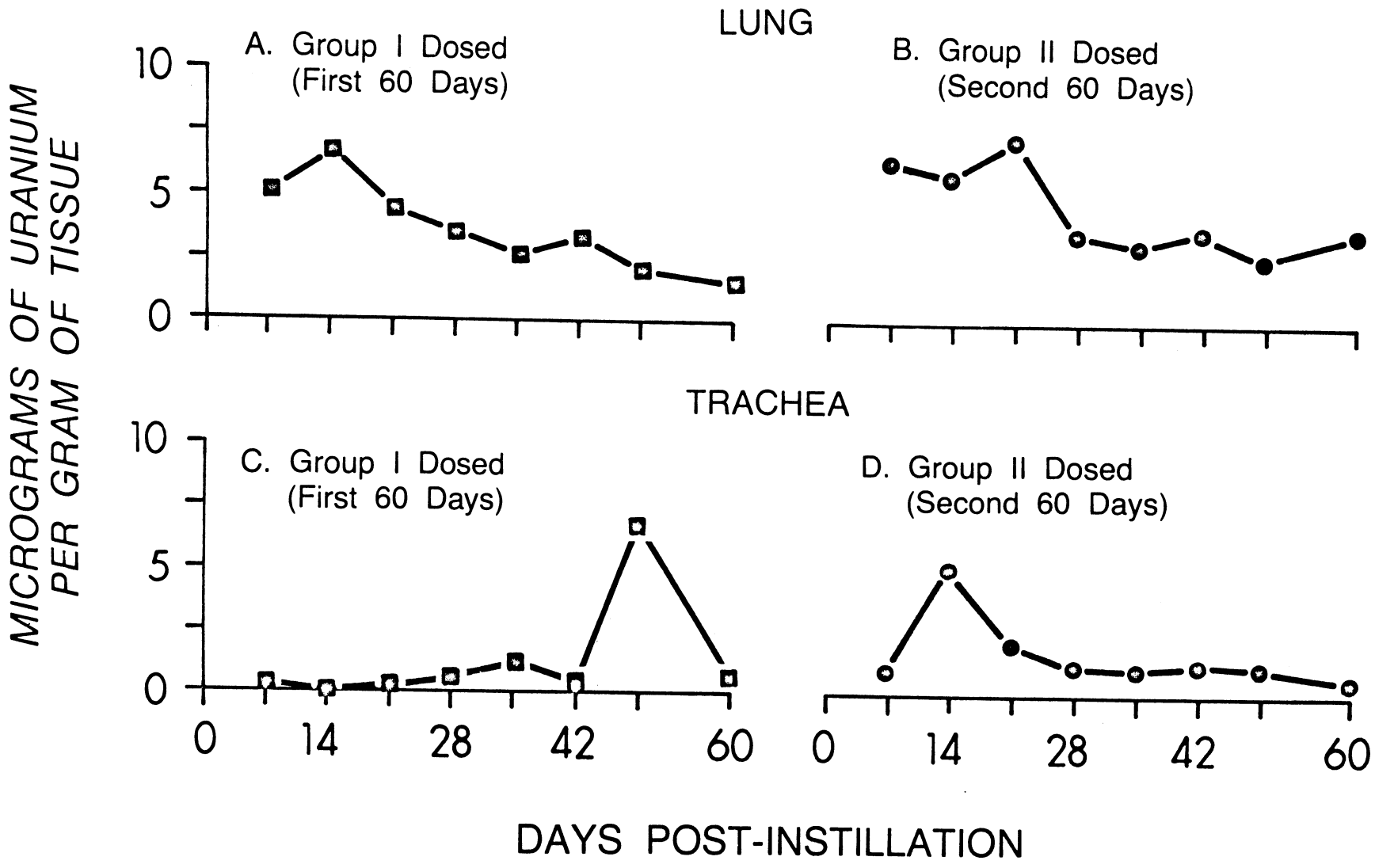

Fig. 49 Retention of uranium in the lung and trachea after one (□) and after two (●) instillations of 2 mg UO_2F_2/kg.

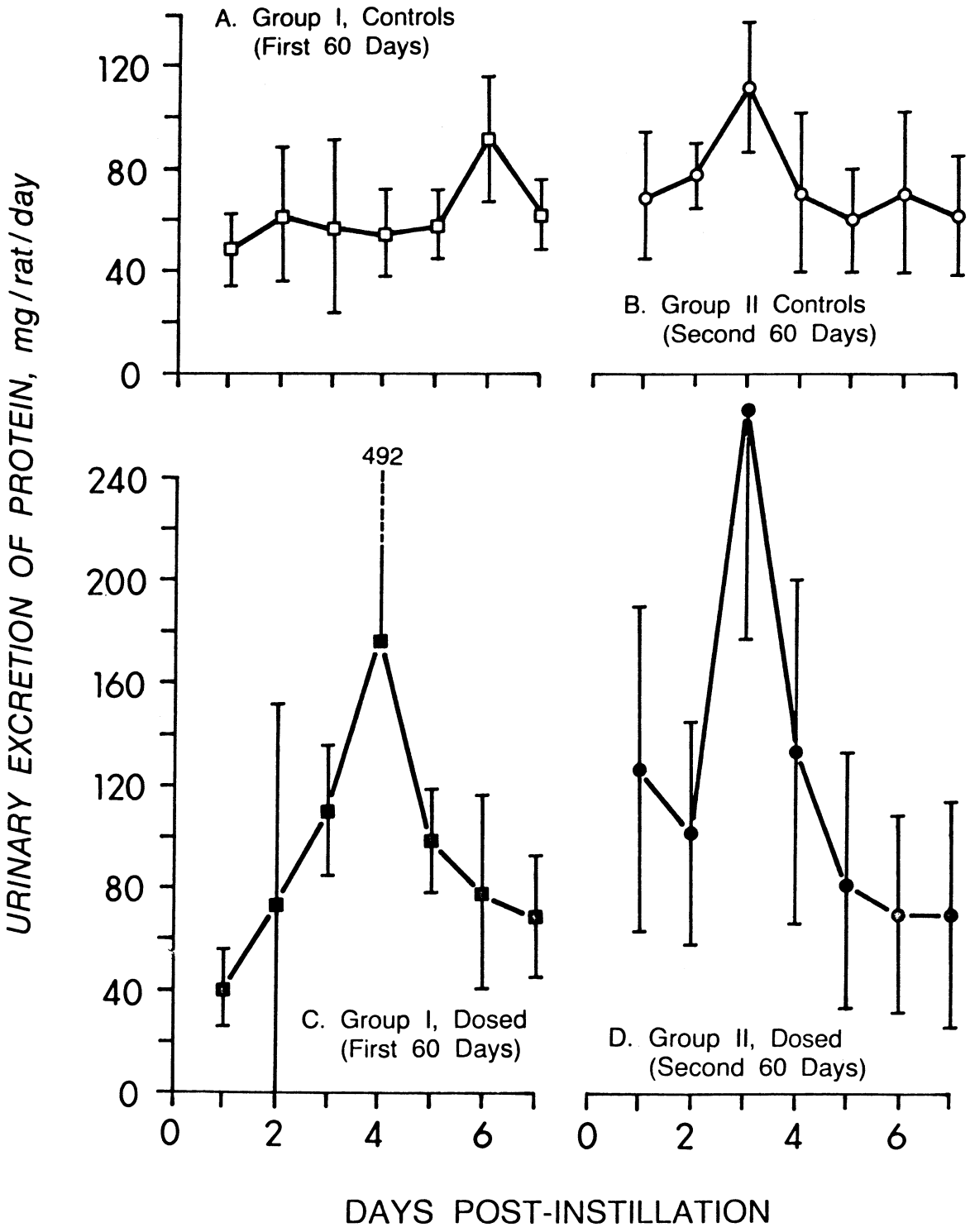

Fig. 50 Urinary excretion of protein during days 1-7 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

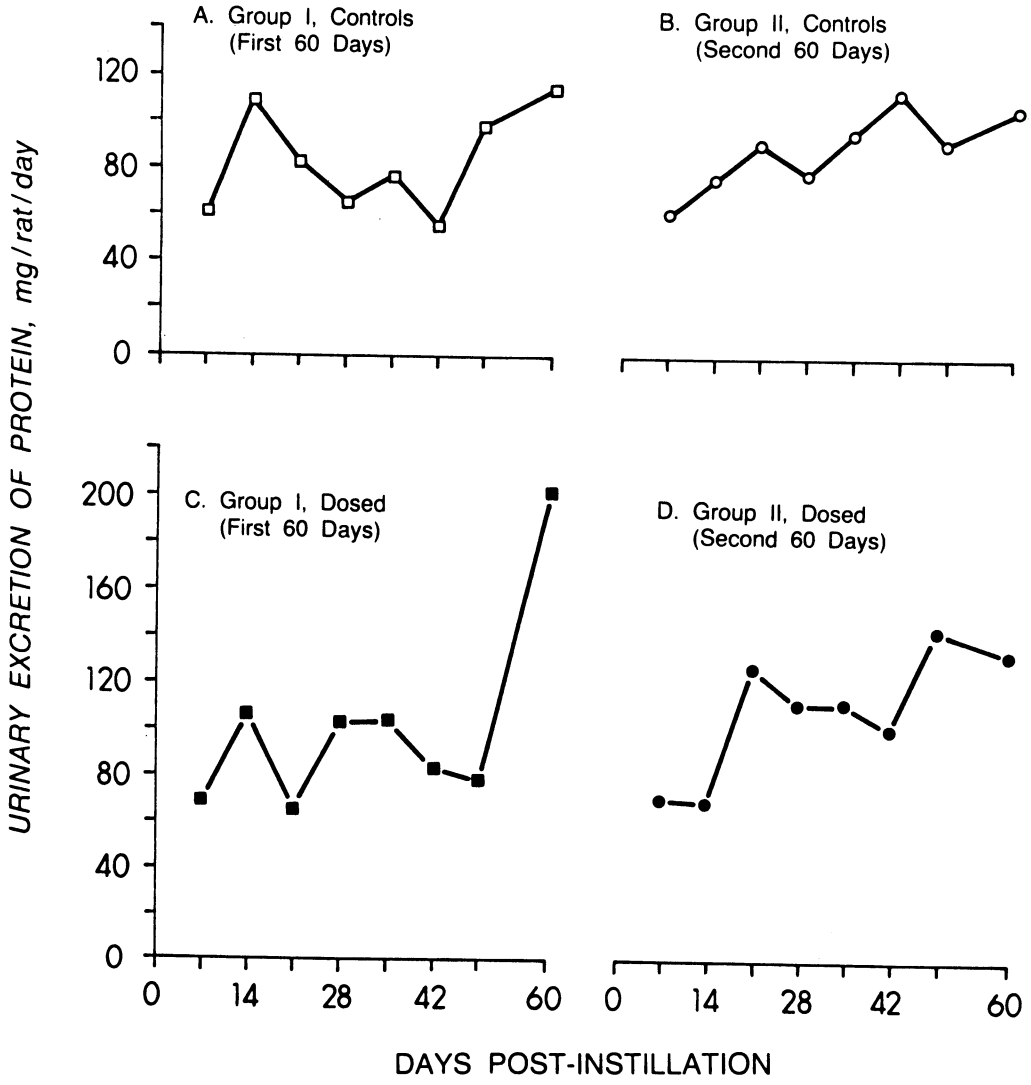

Fig. 51 Urinary excretion of protein during days 7-60 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

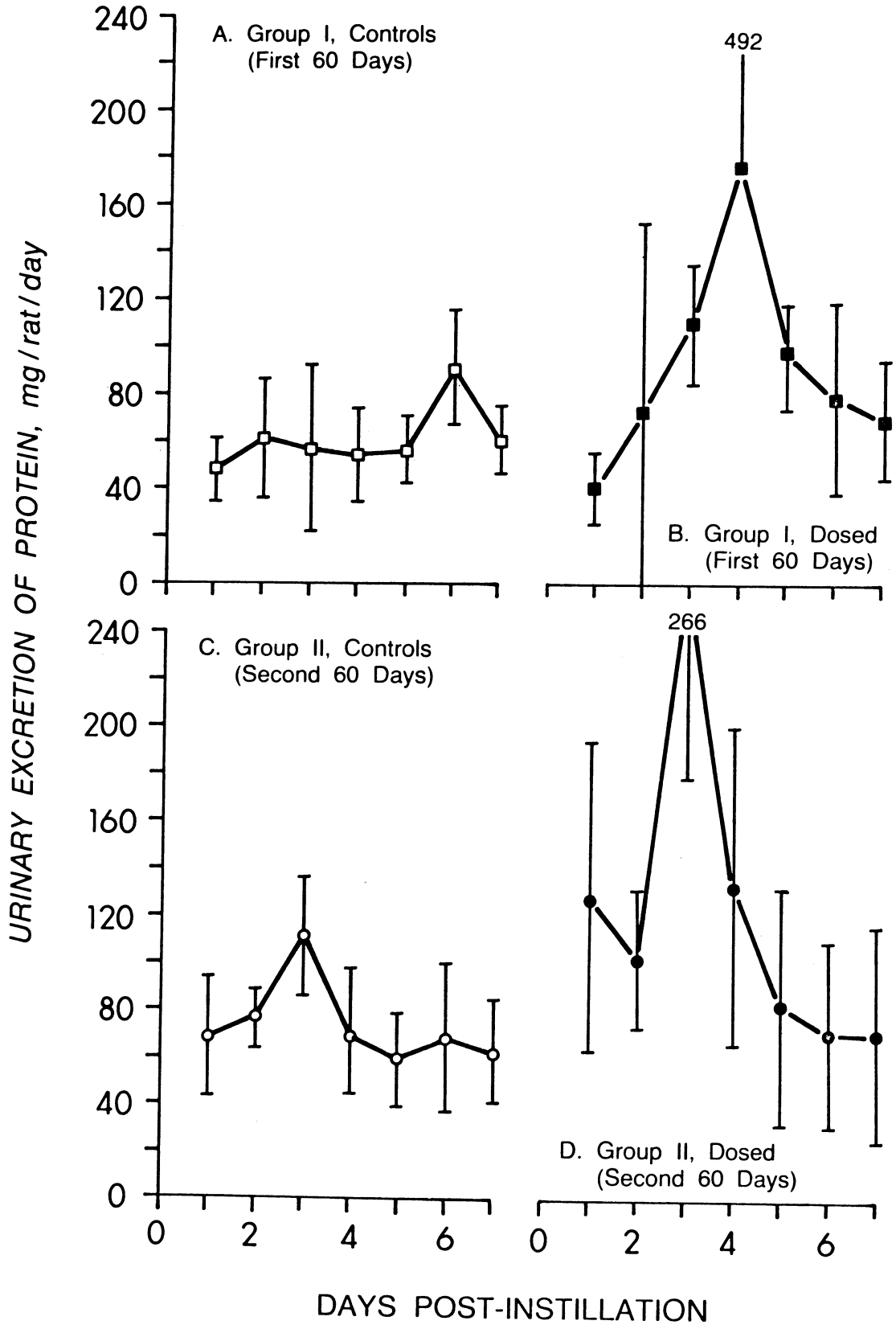

Fig. 52 Urinary excretion of protein during days 1-7 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

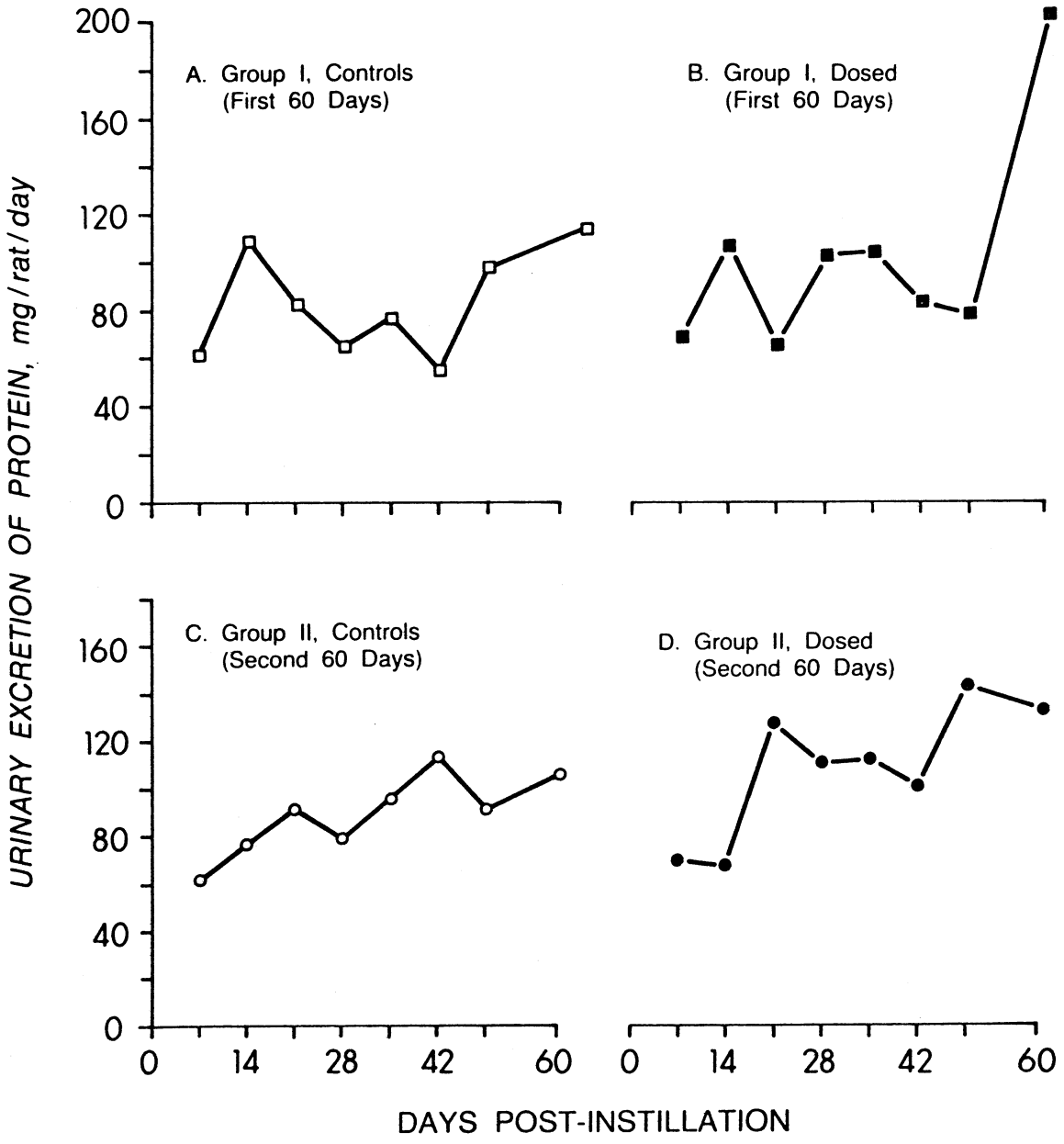

Fig. 53 Urinary excretion of protein during days 7-60 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

b) Plasma Urea Nitrogen: Figures 54A and B, and 55A and B indicate that plasma urea N in the Group I and Group II controls was close to 10 mg per 100 ml plasma throughout the entire experiment. Three days after the instillation of one dose of UO_2F_2 (the earliest sample taken) plasma urea N was elevated (Figure 54C and 56B), and continued to rise for the remainder of the first week post-instillation to a maximum approximately 22 times greater than control levels. The twice dosed animals also showed an increased plasma urea N concentration from day 3 onward; however, the maximal concentration (10-fold) achieved in the first 7 days was appreciably less than that reached after the first administration of UO_2F_2 (Figure 54D and 56D). Figure 55C and D shows that plasma urea N declined from the high concentrations seen 7 days post administration. Concentrations were approximately normal in the once dosed animals 14-28 days after receiving the UO_2F_2 (Figure 57A and B), but in the twice dosed animals the return to normal levels was considerably slower, 49 days being required before plasma urea N was again in the normal range (Figure 57C and D). The maximal concentration reached after the second dose was only about 80 percent of that engendered by the first dose, suggesting a degree of tolerance had been achieved. However, the longer time required to return to normal after the second dose argues against this.

c) Glucose: Excretion of glucose by control rats and by those receiving UO_2F_2 is shown in Figures 58-60. Control rats of both groups usually excreted approximately 10-20 μmoles of glucose daily during the first 7 days after the instillation of Tris buffer (Figure 58A and 1B); excretion was not appreciably different during the remainder of the study, as is shown in Figure 57A and B. Upon administration of the first dose of UO_2F_2, a prompt elevation of urinary glucose was evident 2 days later (Figure 58C). Maximal glucose excretion was seen on day 4, after which the excretion declined. Variability among animals also increased promptly, but was less during the latter part of the week. In the twice dosed rats glucose excretion also increased promptly (Figure 58D). The maximal amount excreted again occurred on day 4 after instillation, but was not as great (490-fold) as was seen after one dose (730-fold). Figure 60A and B indicate that glucose excretion returned to the normal range 14 days after administration of UO_2F_2 in the once dosed rats. The twice dosed rats required an additional week before glucose excretion was again normal (Figure 60C and D). As noted for urea N, the lesser response after the second dose suggests an acquired tolerance, but the slower return to normalcy is not consistent with this.

The promptness with which glucose excretion responds to the instilled UO_2F_2, and especially the magnitude of the response, recommends this determination as a simple, sensitive and early measure of the effects of exposure to soluble uranium.

d) Citrate: Citrate excretion in the urine was measured daily during the first 7 days after each instillation of UO_2F_2, and then once again 14 days after each instillation. The data are shown

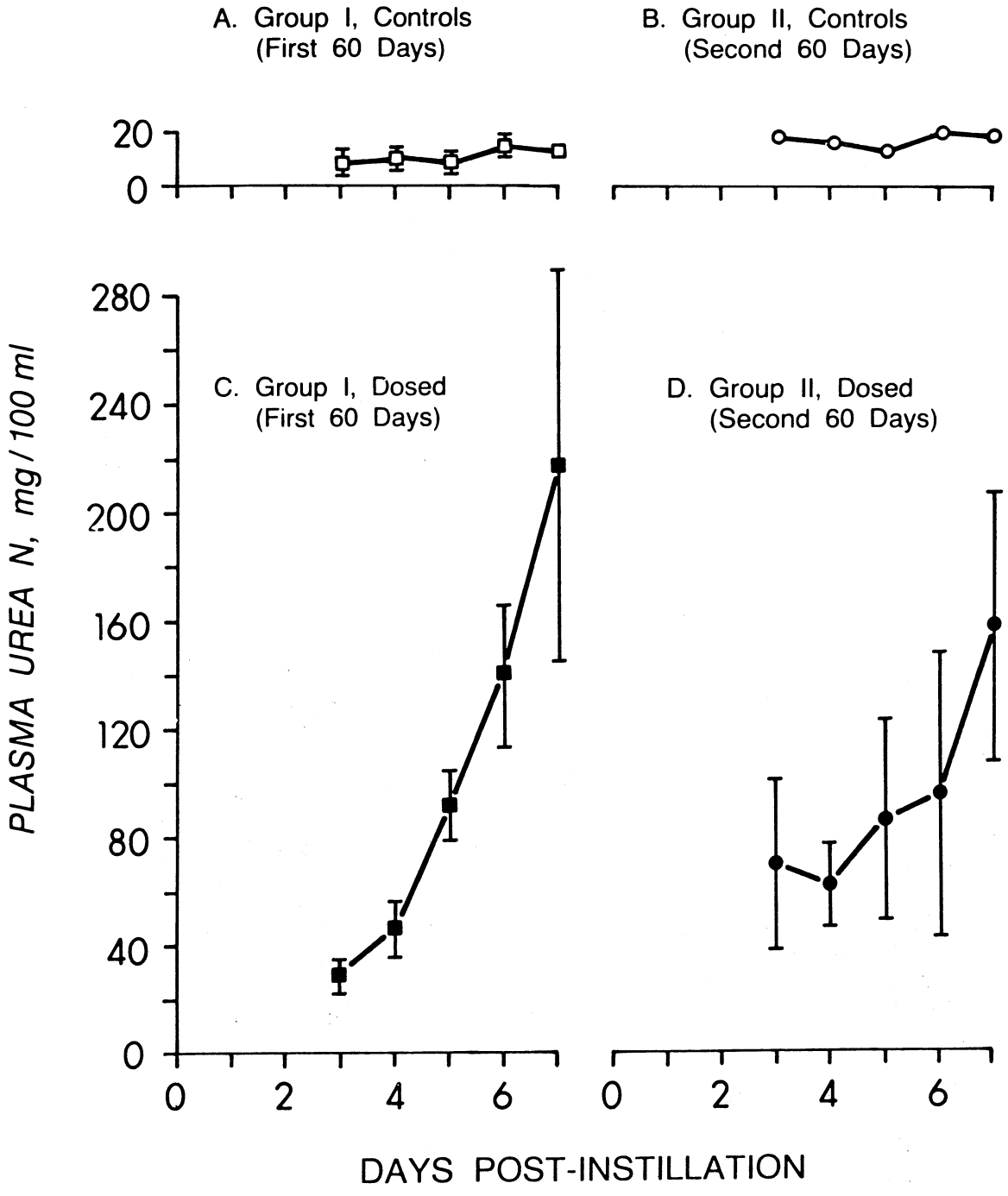

Fig. 54 Plasma urea N during days 1-7 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

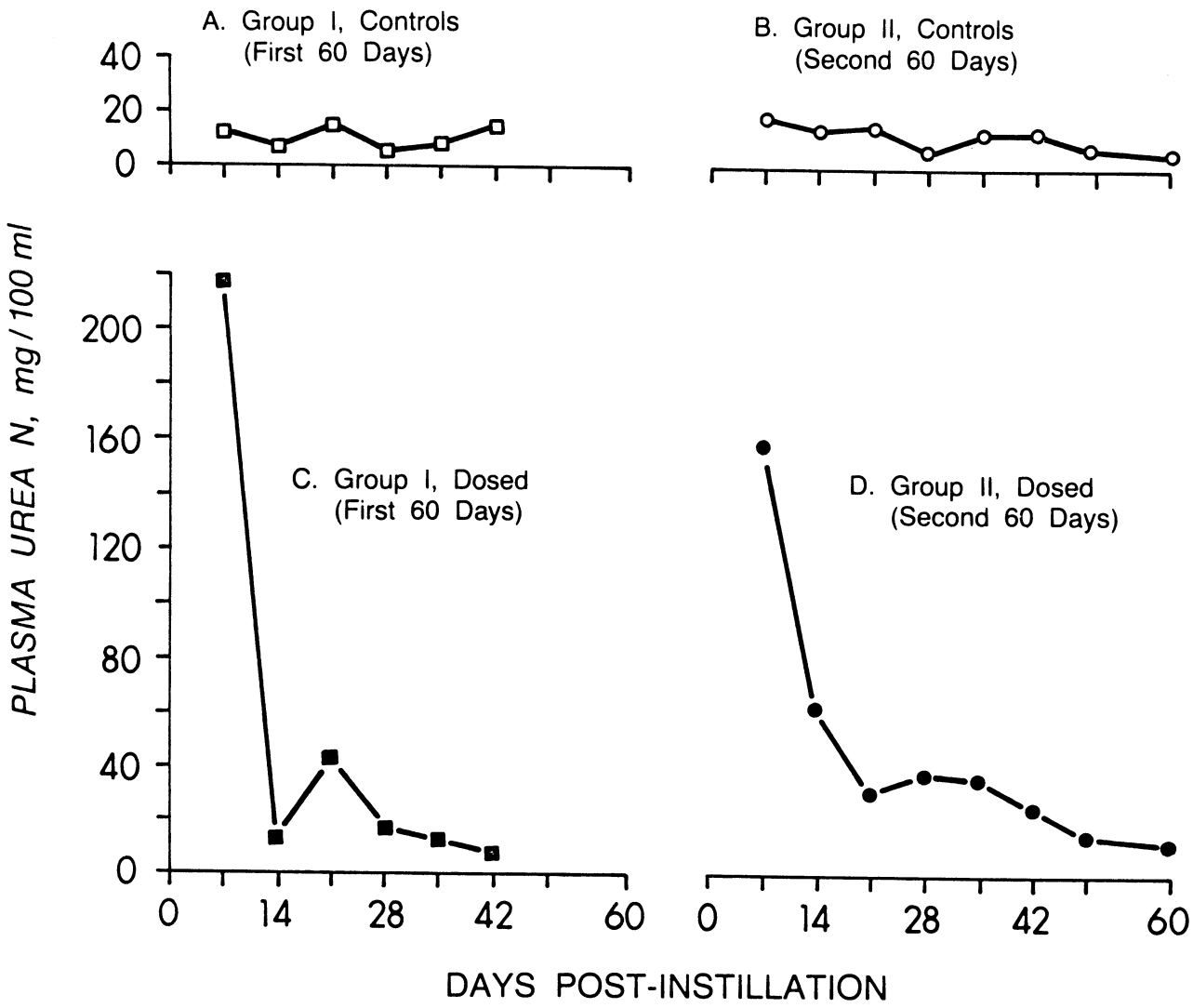

Fig. 55 Plasma urea N during days 7-60 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

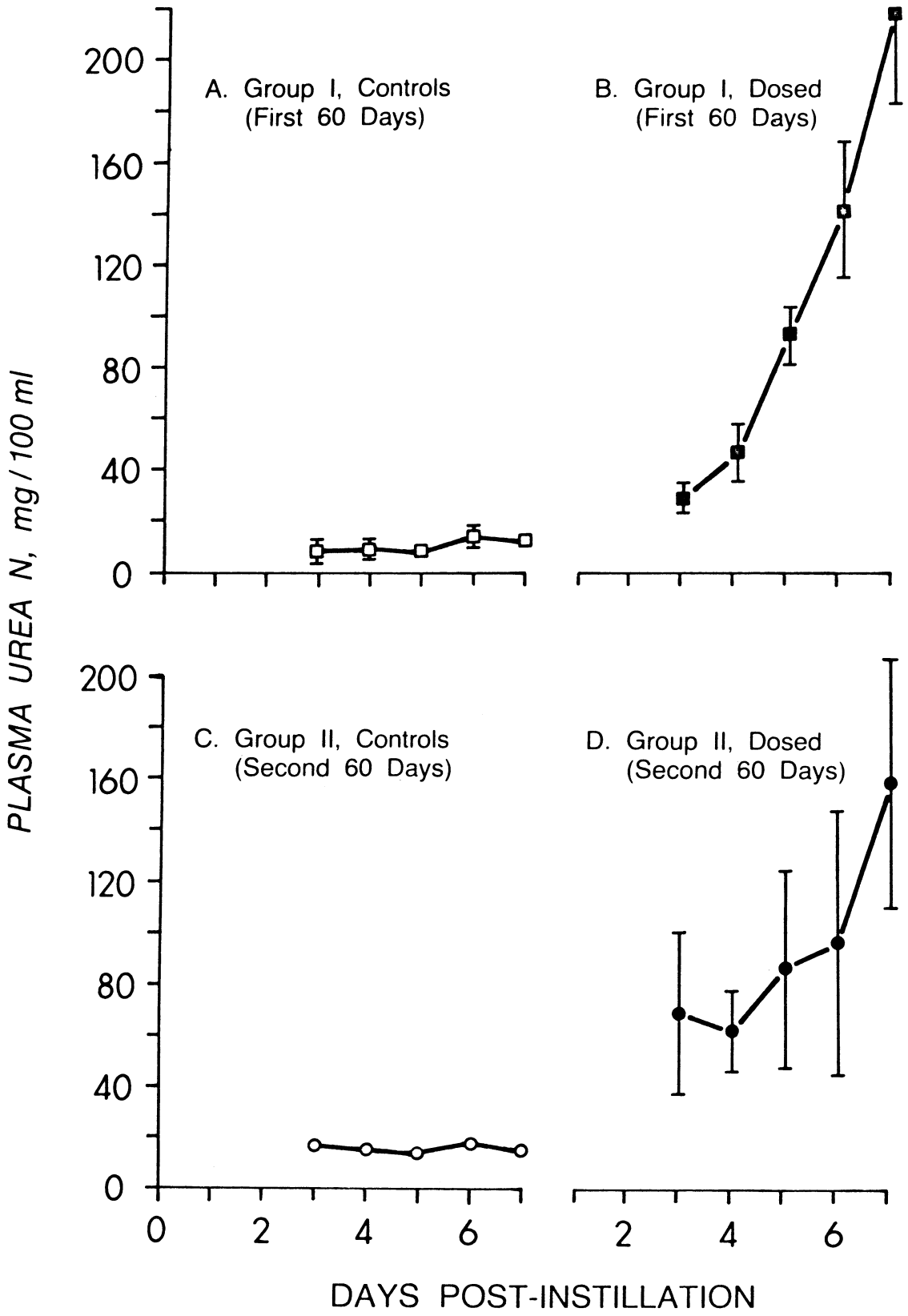

Fig. 56 Plasma urea N during days 1-7 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

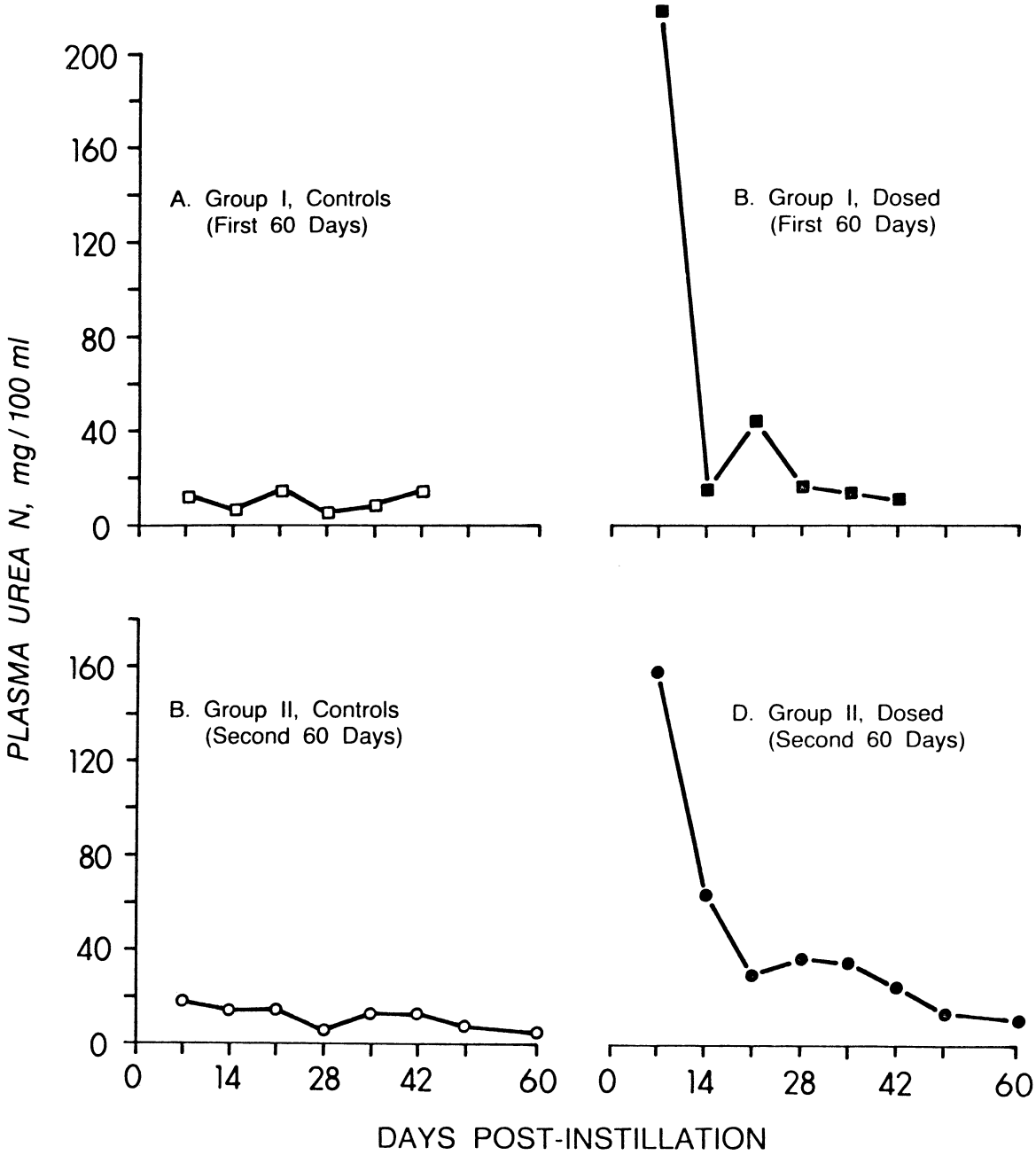

Fig. 57 Plasma urea N during days 7-60 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

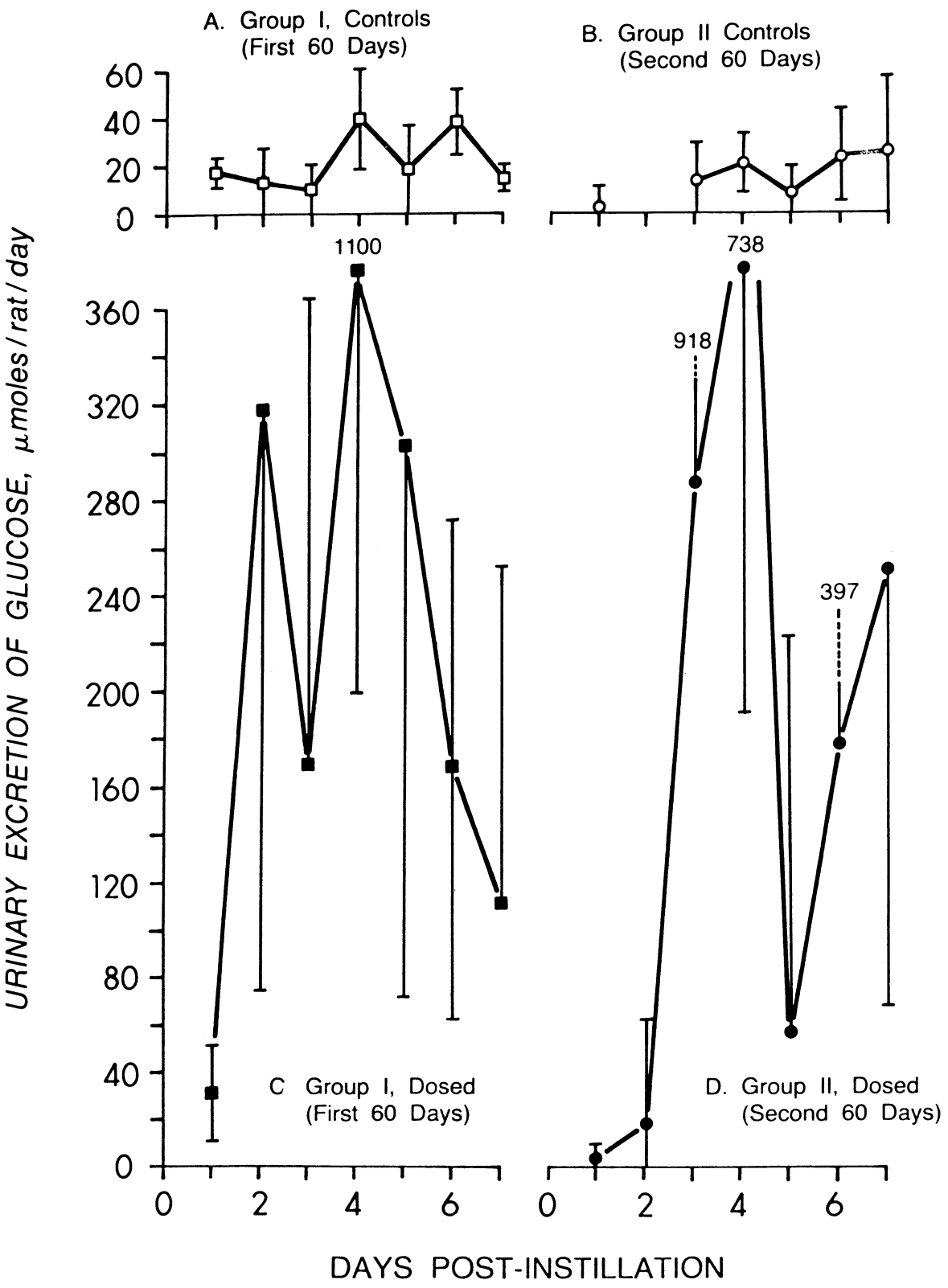

Fig. 58 Urinary excretion of glucose during days 1-7 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

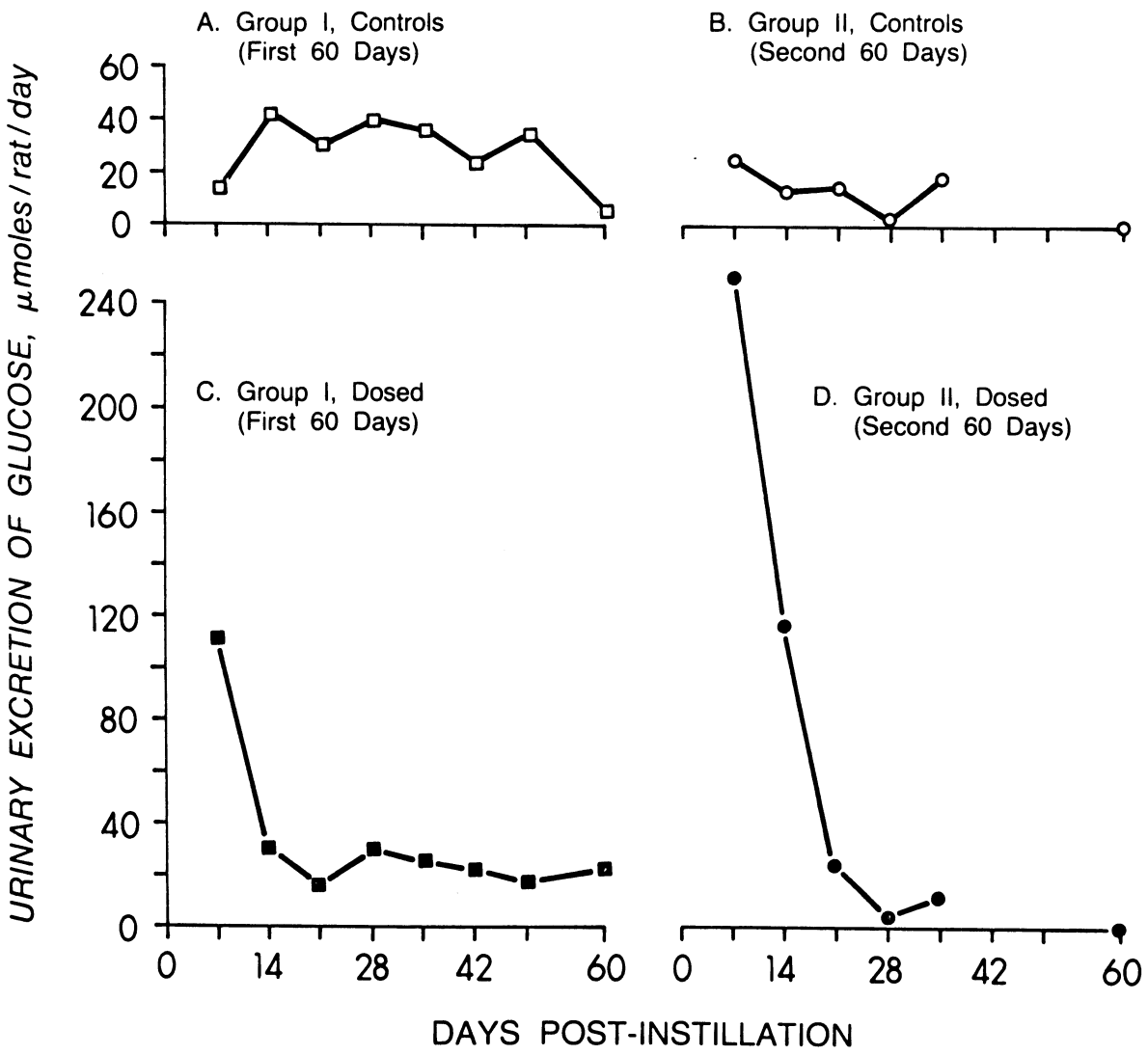

Fig. 59. Urinary excretion of glucose during days 7-60 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

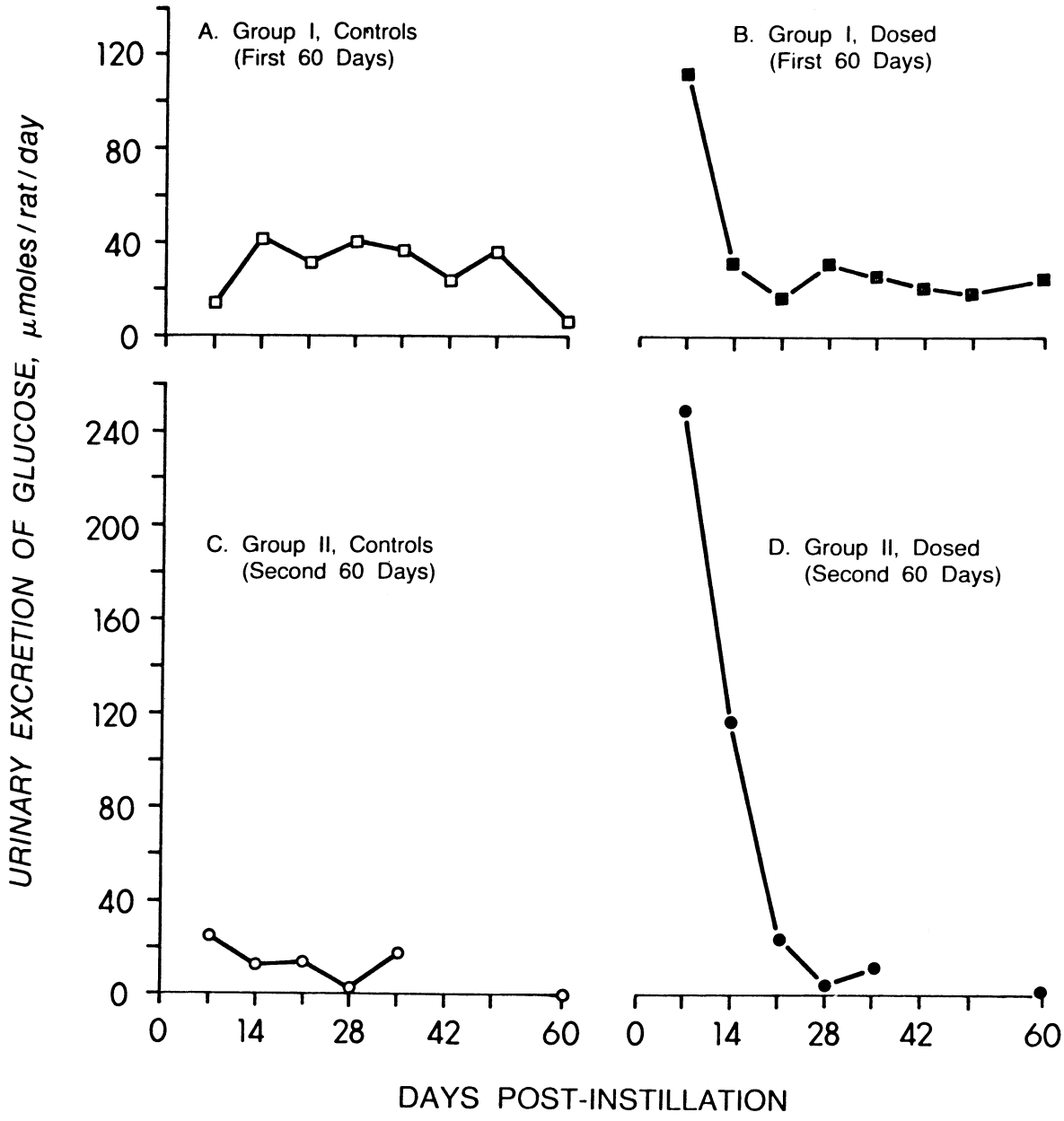

Fig. 60 Urinary excretion of glucose during days 7-60 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

in Figures 61 and 62. Comparison of Figures 61A and B shows that citrate excretion varied between 17 and 27 μmoles per day in the Group I controls, and that it was appreciably less in the Group II controls. Figure 61C and D shows that following either one or two instillations of UO_2F_2, excretion of citrate was greatly reduced during the next seven days. Fourteen days after instillation however, citrate excretion had rebounded to above normal amounts. The decreased excretion of citrate in dosed animals relative to that in control rats is shown also in Figure 62A and B and 62C and D, where excretion in the once or twice dosed animals is placed adjacent to that of its control group. The decline in excretion of citrate appears to be more prompt in the twice dosed rats than in the once dosed animals (Figure 61C and D), though the depression is not more severe. These findings are quite different than those reported by Haven and Randall (1948), who noted progressively greater increases in urinary citrate after multiple doses of uranium, and suggested the availability of citrate, as a complexer of uranium, conferred a tolerance to the renal effects of soluble uranium.

e) Volume: The volumes of urine excreted by control and by treated rats are shown in Figures 63-66. Group I control animals excreted approximately 5 ml of urine per rat per day (Figure 63A), while the Group II control rats, 60 days older, excreted about 10 ml per rat per day (Figure 63B) during the first 7 days post-instillation. During the interval 7-60 days post-instillation control animals of both groups tended to excrete slightly more urine than during the first 7 days, but the increases are not striking (Figure 64A and B). The daily urine volume for dosed rats of either group was approximately normal for the first 2 days after instillation of UO_2F_2, but on day 3 post-instillation the urine volume for both groups increased sharply by a factor of four (Figure 63C and D). This response is shown also in Figure 65A and B and in Figure 65C and D, where a comparison of dosed and controlled animals can be made more readily. Excretion was appreciably more variable among individual animals and from day to day. Figure 63C and D shows that the twice dosed animals excreted slightly more urine per day, beginning at day 3, than did the once dosed animals. However, the difference, if any, is not pronounced. Figure 66A and B, and 66C and D demonstrate that the elevated excretion of urine by dosed rats of either group exceeded that of the respective control animals throughout the 60 days post-instillation. It is also evident that although the difference in excretion between controls and once dosed animals becomes less during the latter 8 weeks of the experiment (Figure 66A and B), the difference never disappeared. In the twice dosed animals (Figure 66C and D) the difference was more pronounced and remained so throughout days 7-60 post-instillation.

Inasmuch as blood fluoride concentrations of 1 μg F/ml or more are now recognized as causing diuresis in rats and in the human, it is reasonable to question whether or not fluoride released *in vivo* from the UO_2F_2 may have contributed to the increased urine volumes noted.

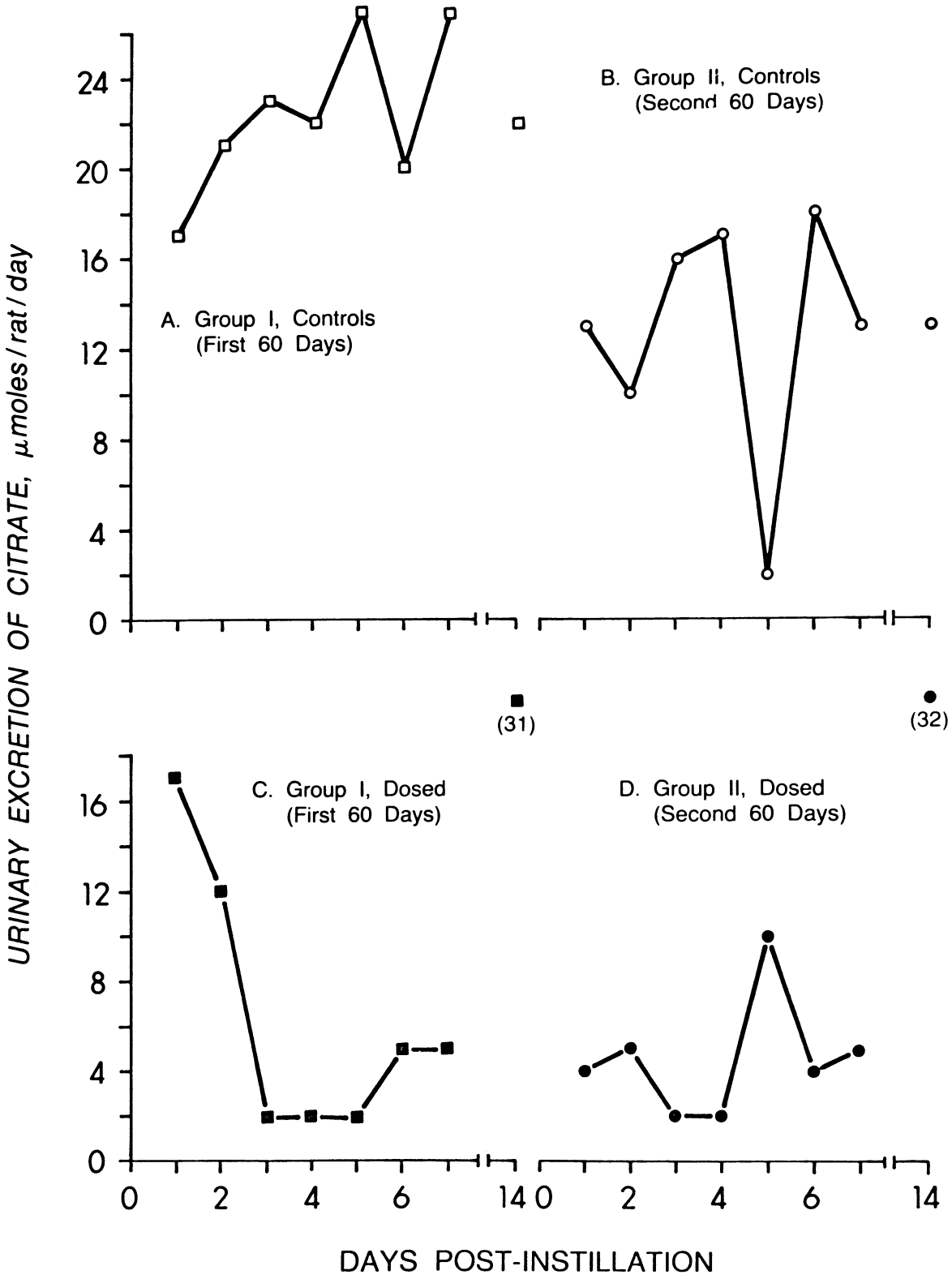

Fig. 61 Urinary excretion of citrate during days 1-7 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

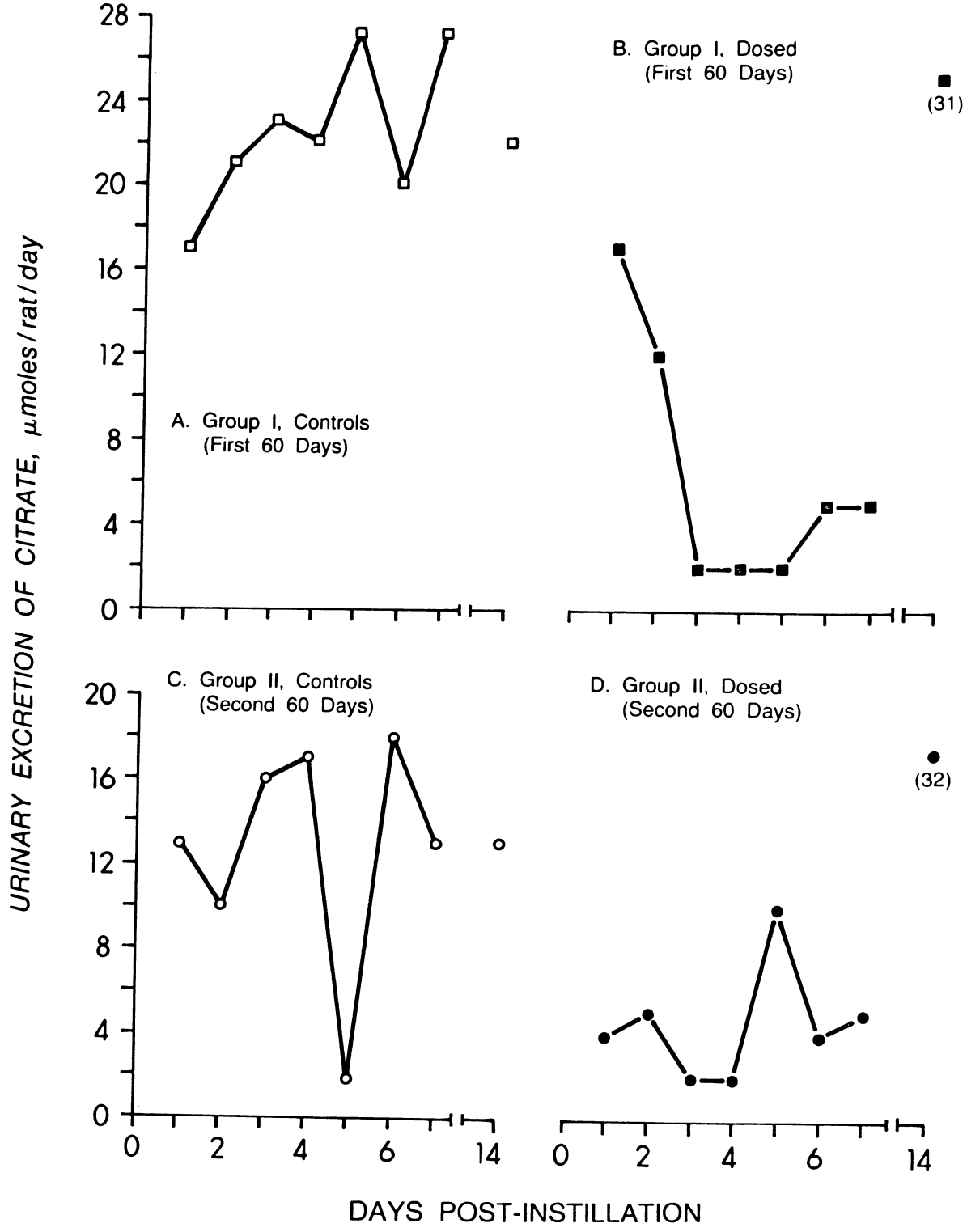

Fig. 62 Urinary excretion of citrate during days 1-7 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

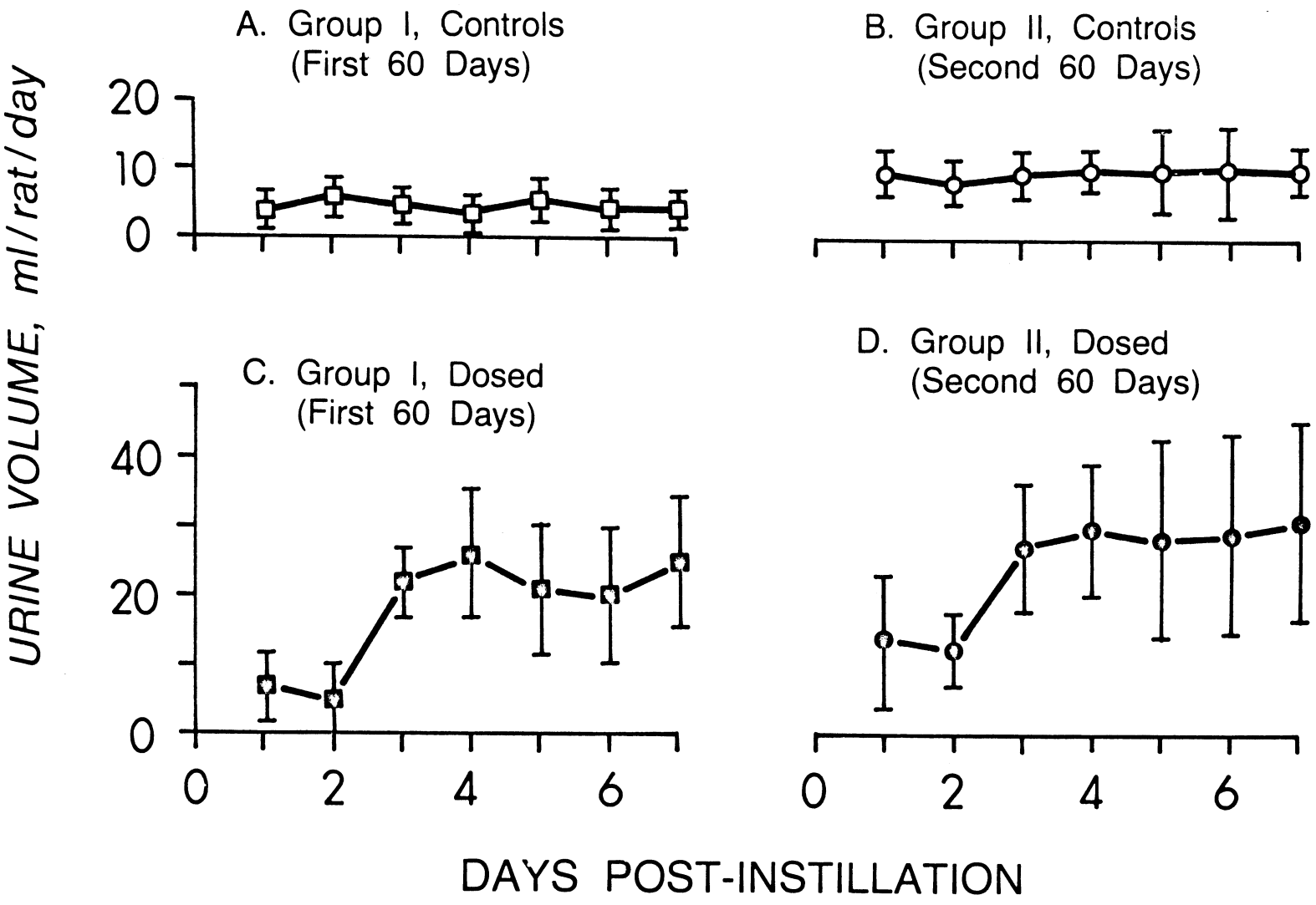

Fig. 63 Daily urine volume during days 1-7 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

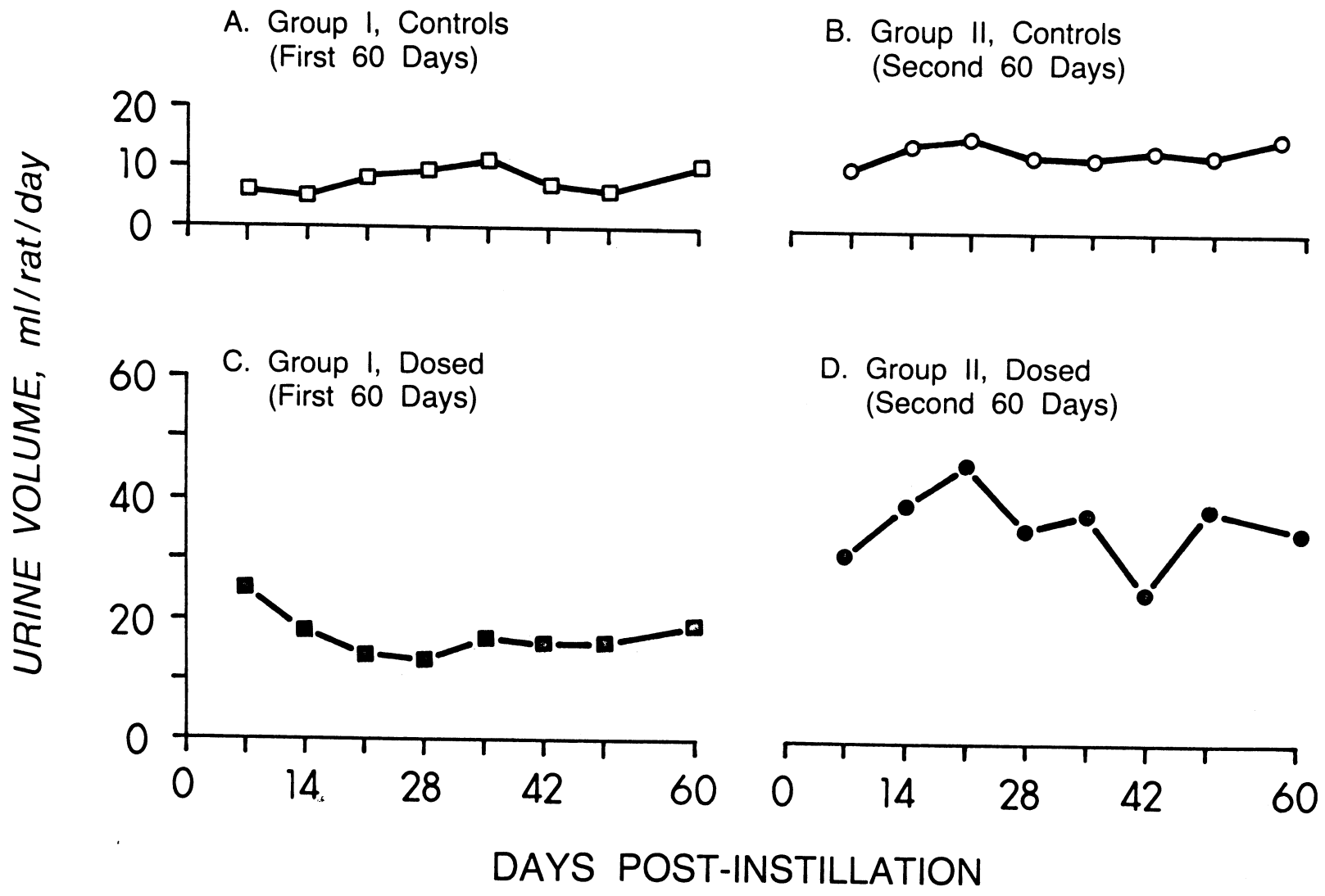

Fig. 64 Daily urine volume during days 7-60 post-instillation of one (controls, □ ; dosed, ■) or two (controls, ○ ; dosed, ●) instillations of 2 mg UO_2F_2/kg.

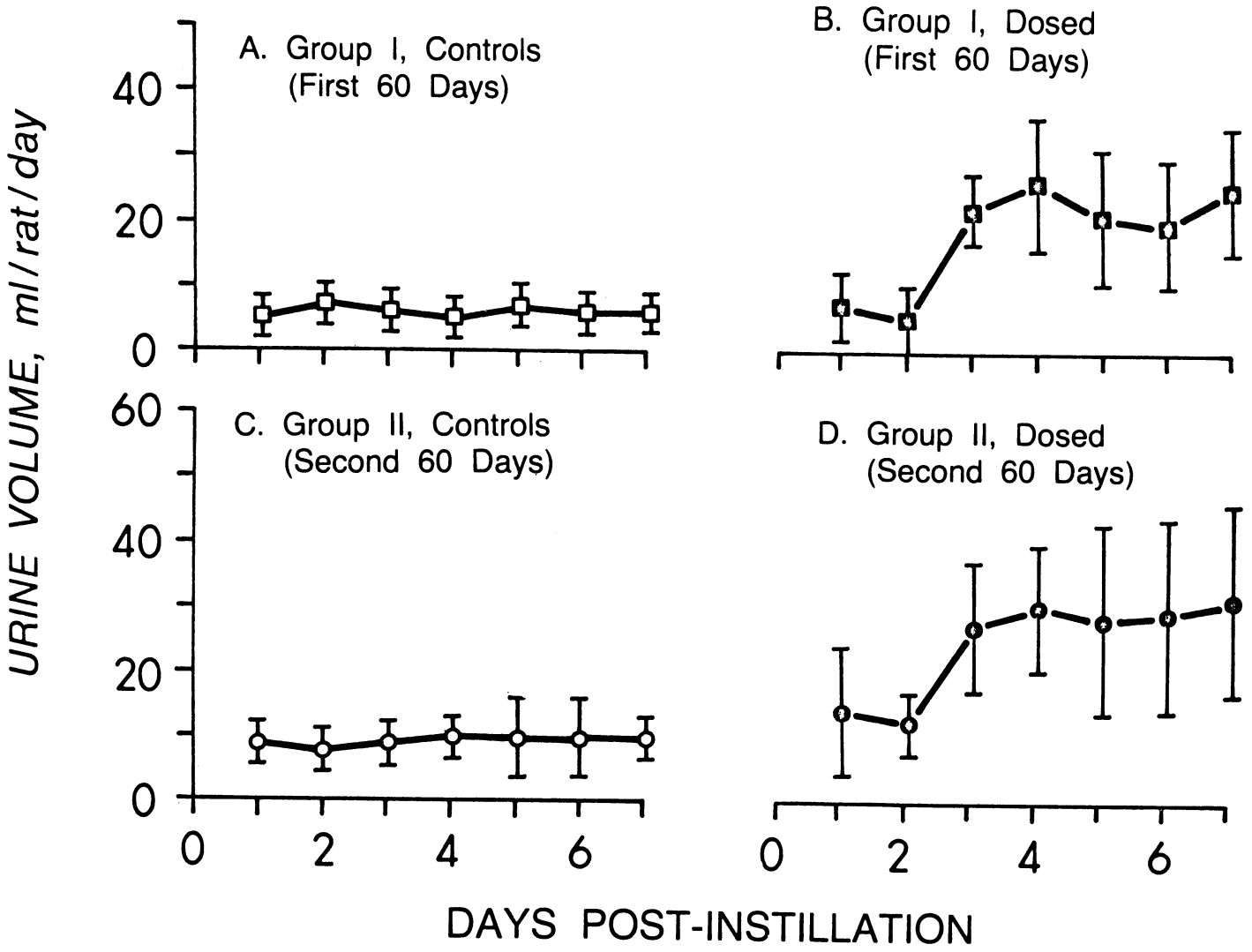

Fig. 65 Daily urine volume during days 1-7 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

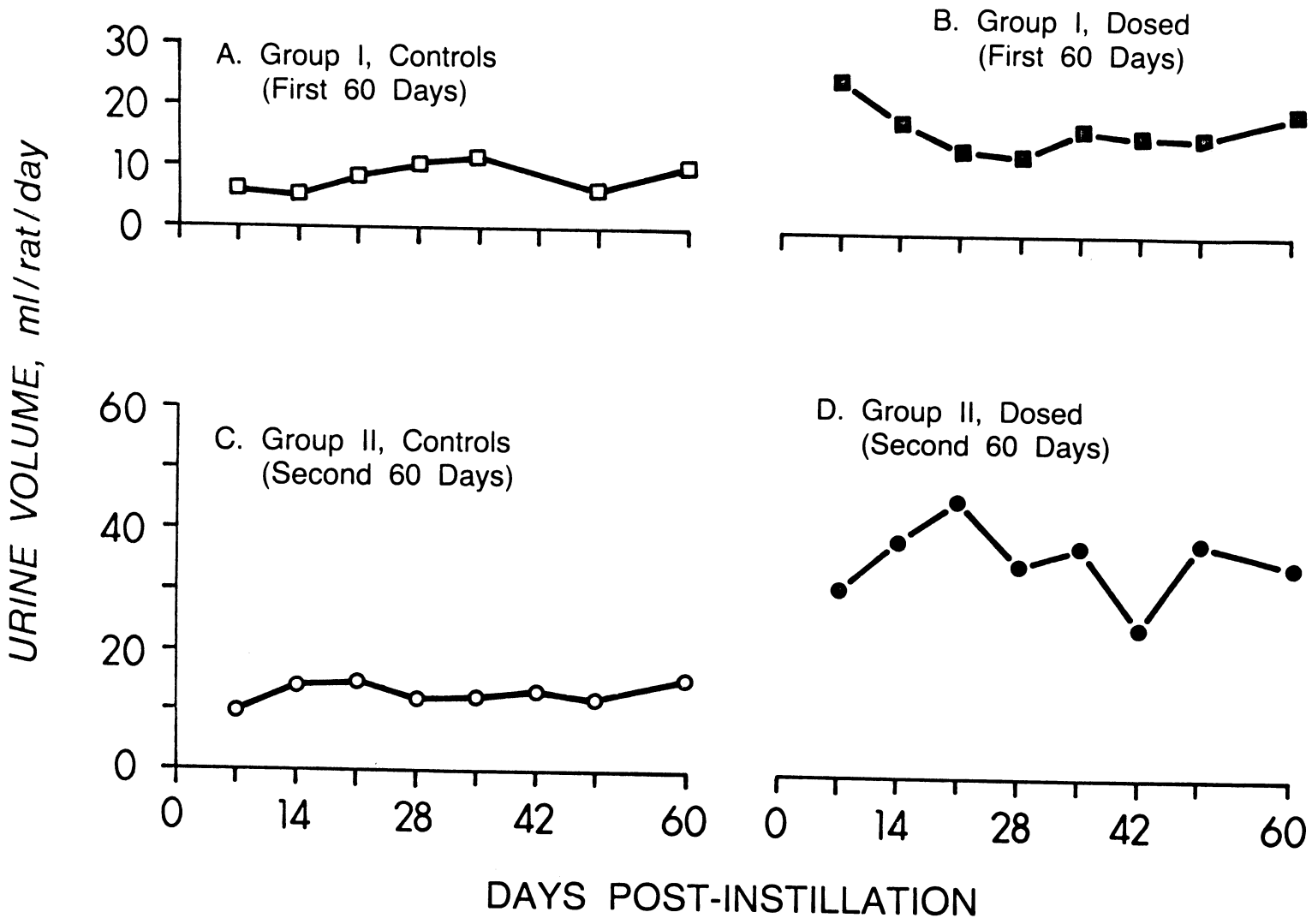

Fig. 66 Daily urine volumes during days 7-60 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

E. Tolerance to Uranium-induced Renal Injury

If tolerance is viewed as the successful adaptation of an animal to successive low level insults, this study cannot be said to have demonstrated a tolerance in rats to the toxic effects of two doses of UO_2F_2 administered 60 days apart. Exceptions to this generalization are suggested by the fact that mortality following the second dose was less than after the first dose, and by the fact that plasma urea N did not increase to the same extent following the second dose. However, a number of measures of renal toxicity indicated that the kidneys of rats receiving the first dose of uranium were still impaired 60 days later, and not capable of functioning as well as those in the naive animal. While plasma urea N did not increase as greatly after the second dose, neither did it return to normal levels as promptly. Urinary excretion of protein and glucose also returned more slowly to normal levels. The volumes of water ingested and urine excreted were elevated and remained so. Histologically evident damage in the kidney was not completely repaired after the first 60 days, and the damage produced by the second dose was more severe and widespread in the organ. The ability of the kidney to excrete uranium was significantly less after the second dose, implying a more rapid buildup of renal burden with repeated exposures. These cumulative renal effects in time may prove to negate the short term reduction in lethality. In essence, effects in rats of an intratracheal instillation of small amounts of UO_2F_2 persist for at least 60 days thereafter, and little or no protection is provided against the effects of a second dose received 60 days after the first.

F. Special Studies

a) Urinary Metallothionein: Inasmuch as metallothionein is known to appear promptly in the urine of cadmium poisoned rats, a limited investigation of the possibility that uranium may behave smilarly, was warranted. Urines obtained from two twice dosed rats 2, 4, 6 and 14 days after the second instillation of UO_2F_2 were assayed for metallothionein by the immunological method of Tohyama and Shaikh (1981). The results are shown in Table 15. Metallothionein was not detected in the control urine. Moreover, it was not detected in 4 of 7 samples from the twice dosed rats; when present, the concentrations were not greatly above the limit of detection. Under the conditions of this experiment, uranium had little effect upon the production of metallothionein.

G. Histopathology

Tissue sections for histological examination were taken at 7, 14, 21, 28, 35, 42, 49 and 60 days after instillation of each of the doses of UO_2F_2.

a) Kidney: At seven days after instillation of the first dose of UO_2F_2 there was persistent severe, acute tubular necrosis involving predominantly Henle's loop and the distal convoluted

Table 15. Urinary excretion of metallothionein by uranium poisoned rats

Days post-instillation	Nanograms metallothionein per ml*		
	Control	Rat No. 52	Rat No. 53
1	ND**		
2		ND	198
4		lost	ND
6		180	158
14		ND	ND

*detection limit, 150 ng/ml

**ND, not detected

tubules. Thirty to 50 percent of the inner half of the cortex was involved, with focal extensions to the capsule. Slight regenerative changes were noted. After 14 days the second sacrifice revealed that only a few tubules still contained necrotic cell debris, while some were filled with hyaline protein casts. Regenerative changes were now widespread and were characterized by flattened to swollen cells with reduced cytoplasm and large nuclei. Many tubules were somewhat dilated while others were small and lined by basophilic hyperplastic cells. At 28 days post-instillation and thereafter the regenerative process appeared to be less active and at later periods residual areas of damage occurred as wedges of compression with glomeruli and tubules which were small and close together and stroma was relatively increased and focally infiltrated with chronic inflammatory cells. While at any given time after exposure the degree of damage including that to glomeruli is similar where present, there was some variation in the extent of such damage from animal to animal.

In animals receiving the second intratracheal instillation of UO_2F_2 and sacrificed on the same schedule thereafter, the changes were qualitatively similar to those seen after the initial dose, but of considerably greater extent, so that at 60 days post-instillation the widespread wedges of atrophy resulted in surface irregularity and a reduction in kidney size. Residual changes from the first exposure were seen in some rats, but tended to be largely obscured by the superimposed alterations. The kidneys of some but not of all rats showed varying degrees of hydronephrosis 28 days after dosing, but its significance and relationship to the experimental procedure was not clear.

It is concluded that the kidneys of rats given a single intratracheal instillation of 2 mg UO_2F_2 kg^{-1} body weight did not return to normal, histologically, in the following 60 days and that the second instillation of the same dosage 60 days after the first, caused more widespread changes so that in the further 60 days less of the renal parenchyma appeared completely functional. These observations are consistent with certain of the biochemical changes noted after the second dose, e.g. increased urine output, increased excretion of protein, and the slower return to normal of urinary glucose and blood urea N.

b) Lung: In the single dose control animals acute focal pneumonitis and focal collapse was seen in the lungs of one rat. Two other control animals showed evidence of acute and/or chronic pneumonia. Dosed animals showed acute pneumonitis in one instance, chronic focal pneumonitis in 3 instances, and minimal focal pneumonia in 4 instances. Atelectatic abscesses were evident in 2 animals, and atelectasis in another instance. There appeared to be no pattern in the incidence of any of these changes. Of 21 dosed animals, normal lungs were seen in 16.

In the twice dosed rats, lungs of the 10 control animals all showed a normal appearance. Lungs of the animals receiving the second

instillation of UO_2F_2 showed focal chronic pneumonitis in 3 instances and focal pneumonia in 3 instances. The lungs appeared to be completely normal in 16 of the 22 twice dosed rats.

There is no evidence in any of the foregoing findings to suggest that the intratracheal administration of either one or two doses of UO_2F_2 had any significance effect upon the morphology of these rat lungs.

c) Trachea: Sections of trachea were taken from control and dosed rats to establish if the administration procedure traumatized this structure. All sections presented a normal histological appearance.

H. Other Assessments

a) Mortality: A total of 51 animals received the first instillation of UO_2F_2. Of these, 6 rats died, corresponding to 11.8 percent of those treated. One animal died 3 days post-instillation, one each on days 6 and 7, and the remaining 3 on days 8-13. The second instillation of UO_2F_2 was given 60 days later to 24 animals. One rat died 10 days later, and one more died 12 days after instillation. Mortality after the second dose thus was 8.3 percent. The initial dose of UO_2F_2 may have generated some degree of tolerance in these rats to a second dose of UO_2F_2, in terms of lethality, inasmuch as percentage mortality after the second dose was only about two thirds of that experienced after the first dose. Half of the deaths after the first dose occurred within the first week, the remainder in the second week. The 2 deaths following the second dose both occurred in the second week post-instillation.

b) Food consumption: Comparison of the two control groups (Figure 67A vs B) indicates no significant difference in daily food consumption. The controls for Group I and for Group II usually ingested between 15 and 20 g of food daily during the first 2 days after intratracheal instillation of the Tris buffer. The first instillation of UO_2F_2 (Figure 67C) decreased food intake to 7-12 g day^{-1} for Group I during the first week, and in Group II which received a second instillation of UO_2F_2 60 days after the first instillation, food intake was 5-12 g per day (Figure 67D). The plots have been regrouped in Figure 68 to permit easier comparison of dosed Group I and dosed Group II rats with their respective controls. From Figures 67 and 68 it is evident that Group II controls, though they are 60 days older than Group I controls, do not differ in daily food intake from the Group I animals. It is also evident that the intratracheal instillation of UO_2F_2 appears to reduce the daily food intake during the first week, relative to controls (Figure 68A vs B; Figure 68C vs D). The daily intake of the twice dosed animals (Figure 67D) does not differ from that of the once dosed rats (Figure 68C). It is also evident that there is considerable spread in the data for each group, and that, though trends are suggested, the differences are not significant.

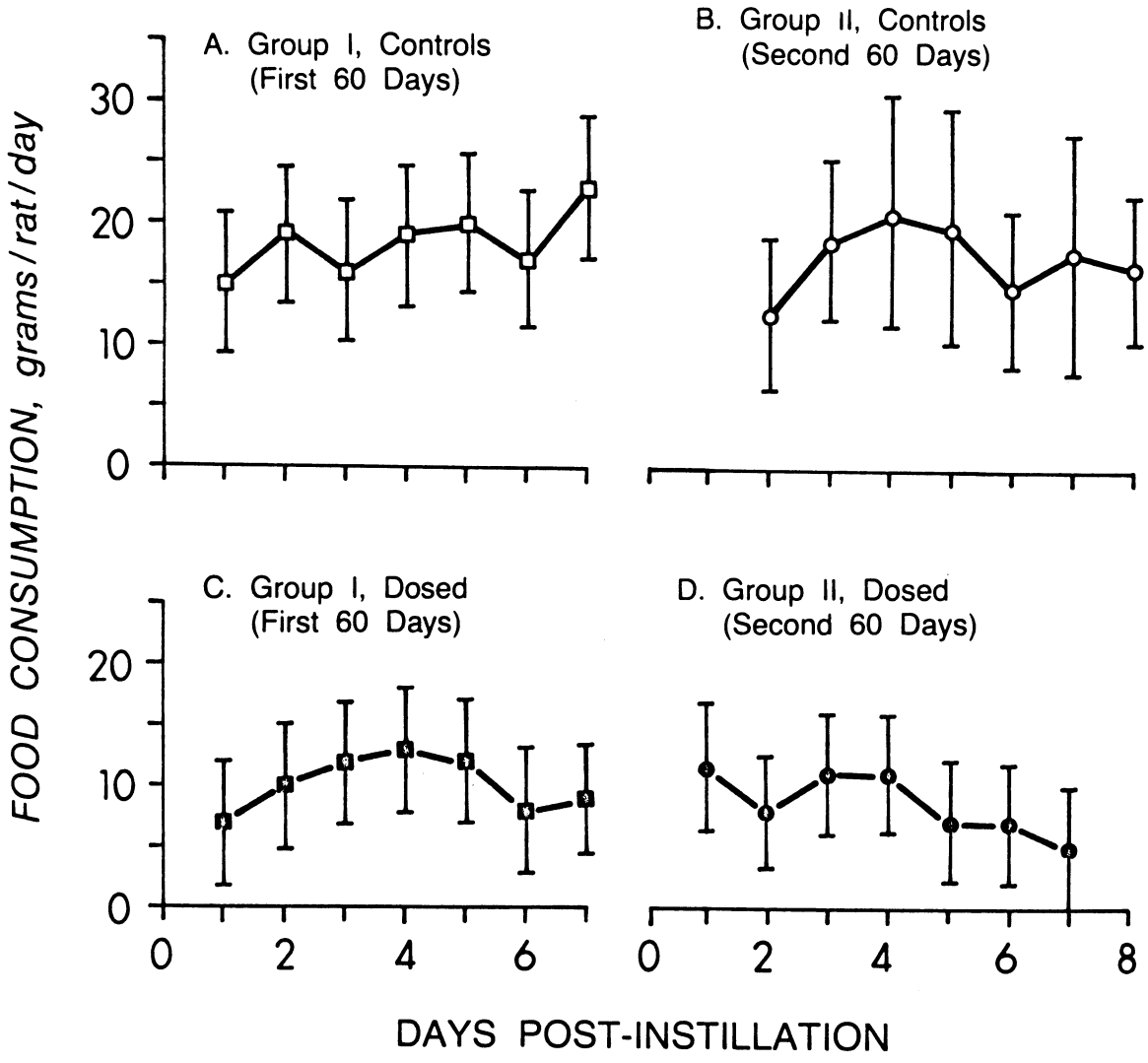

Fig. 67 Daily food consumption during days 1-7 post-instillation of one (controls, □ ; dosed, ■) or two (controls, ○ ; dosed, ●) instillations of 2 mg UO_2F_2/kg.

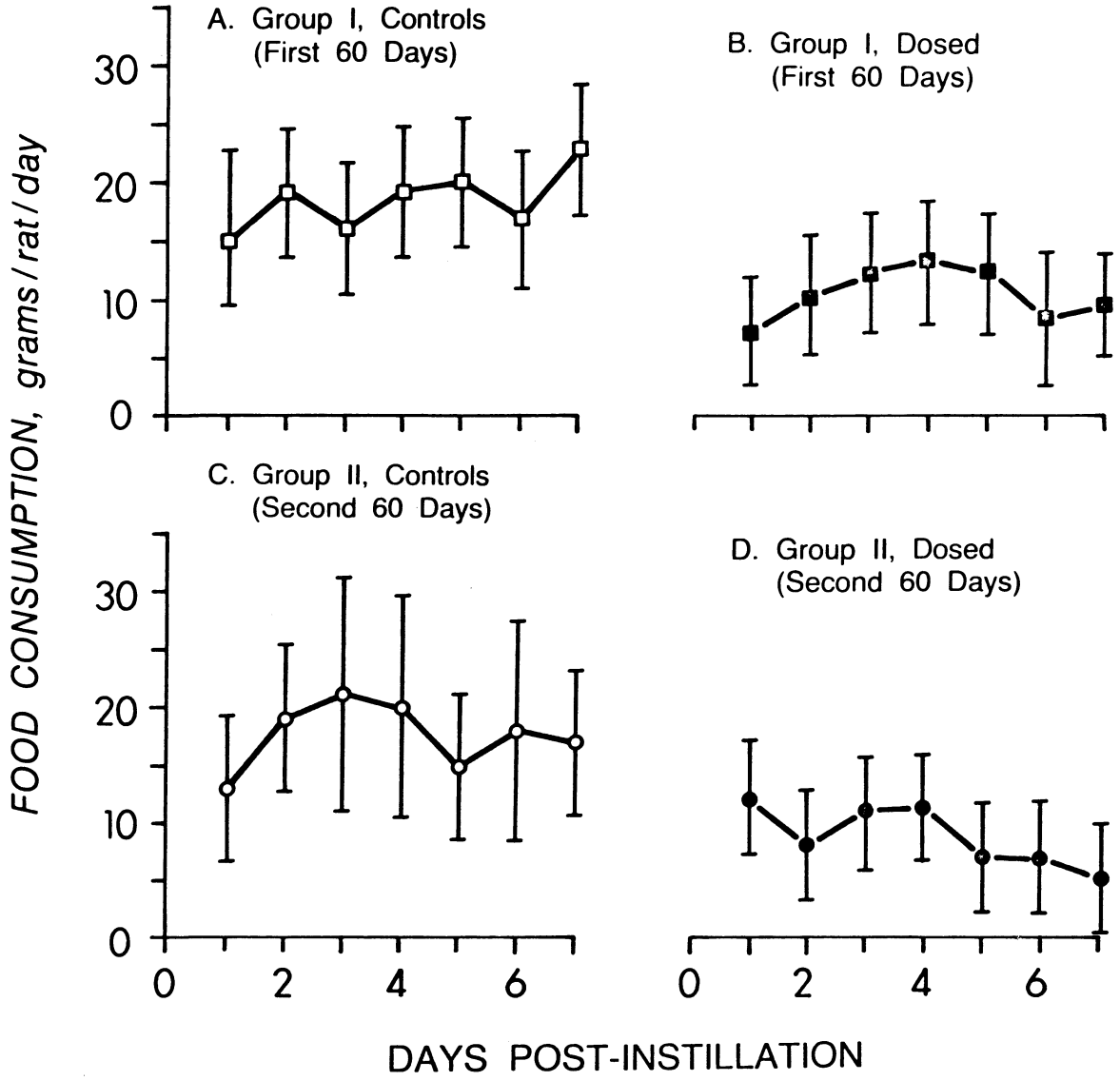

Fig. 68 Daily food consumption during days 1-7 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

Food ingested daily by the control rats of Groups I and II during days 7-60 post instillation are shown in Figure 69A and B, respectively. Each control group ingested 15-23 g of food daily. No pronounced trend in food consumption is evident in either group, nor does the daily amount eaten differ appreciably during this much longer interval from that eaten during the first week. Figure 69C and D shows that a second instillation of UO_2F_2 did not alter the pattern of food ingestion compared to the effect of only one dose. Figure 69C and D also show that food intake was still depressed 7 days after the first or second instillation of UO_2F_2, but that the daily intake recovered to control levels by 14 days post-instillation and remained in the normal range thereafter. This is seen more readily in Figure 70B vs A and 70D vs C.

c) Water consumption: Volumes of water consumed by control and treated animals are presented in Figures 71-74. Figure 71A and B show that during the first 7 days after either the first or second instillation of Tris buffer only, each rat ingested approximately 25 ml of water each day. Following the first instillation of UO_2F_2 the volume ingested increased by the third day to 35 ml per day and remained elevated for the remainder of the week (Figure 71C). The twice dosed rats showed the same pattern of response, with the increase being slightly greater for days 3-7 (Figure 71D). These relationships are shown also in Figure 72A and B, and 72C and D. Considerable variability among animals is evident, which is seen to be greater in those receiving UO_2F_2. Group I control rats showed some increase in daily water ingested during the latter part of the post-instillation period (Figure 73A); Group II controls (Figure 73B) during this latter period showed very little difference from their daily intake during the first week. Figure 73C and D shows that the twice dosed animals consumed appreciably more water than did the once dosed rats, and that this greater intake was maintained throughout the 60 days following the second instillation of UO_2F_2. It is evident from Figure 74A and B that during the interval 7-60 days after the first instillation of UO_2F_2, the water intake of the dosed rats did not differ appreciably from that of control animals. However, the twice dosed rats consumed appreciably more water throughout the 7-60 day interval than did their controls. The differences in amounts of water ingested by the once and twice dosed rats are paralleled by the volumes of urine excreted by these animals (Figs. 65 and 74).

d) Body weight: Figures 75 and 76 present body weight data obtained for the two groups. In these figures the plots represent varying numbers of animals at different intervals, as follows: Group I controls, 8 at day 0 and 1 animal at each point thereafter; Group I dosed animals, 21 rats at day 0, 2 at days 7-35, 3 at day 42 and 4 at days 49 and 60; Group II controls, 10 rats at day 0, 1 at days 7-42 and 2 at days 49 and 60. With these qualifications of the data in mind, it is apparent (Figure 75A and B) that the control animals in both groups increased in body weight with increasing age, and that the Group II control rats tended to be a little heavier

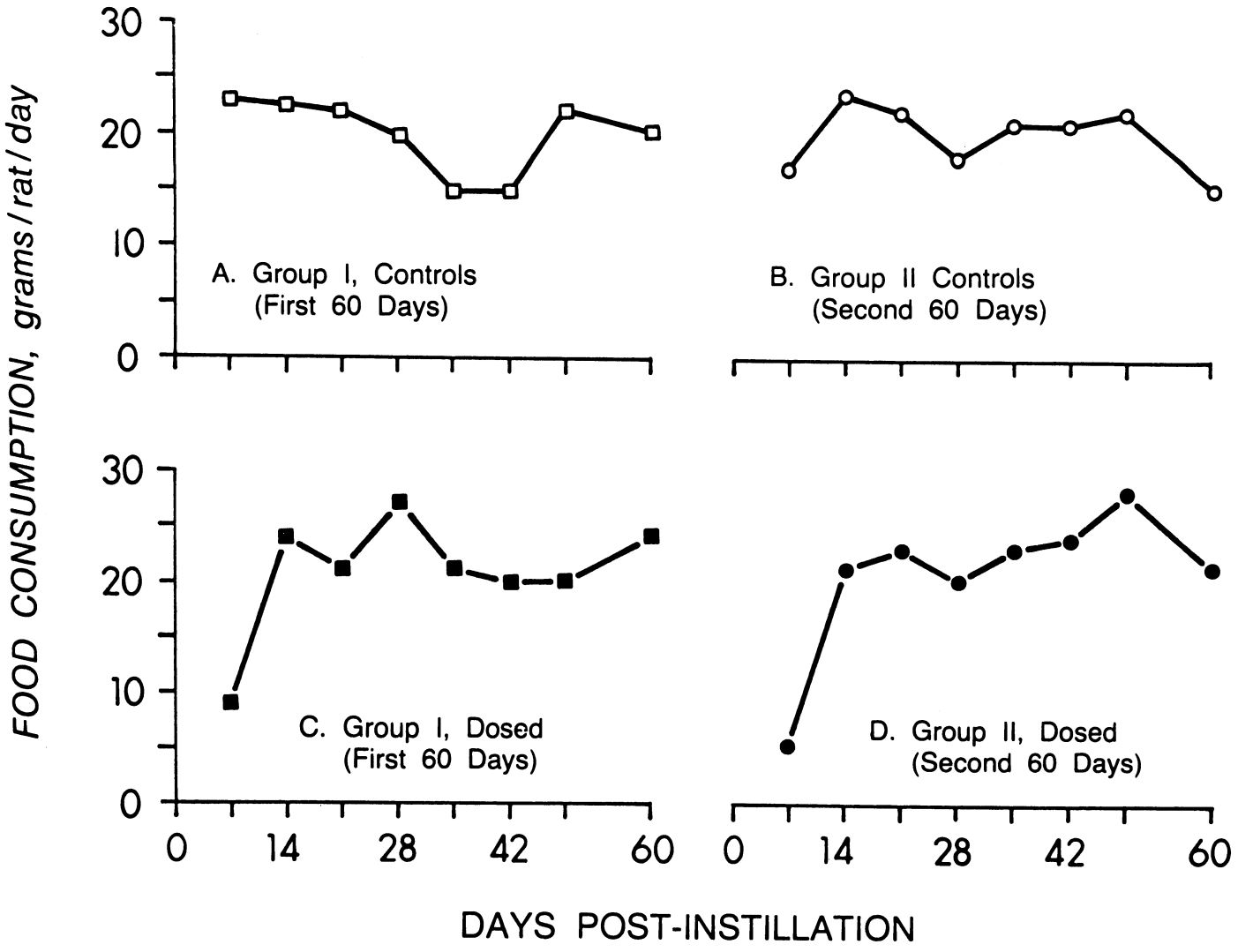

Fig. 69 Daily food consumption during days 7-60 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

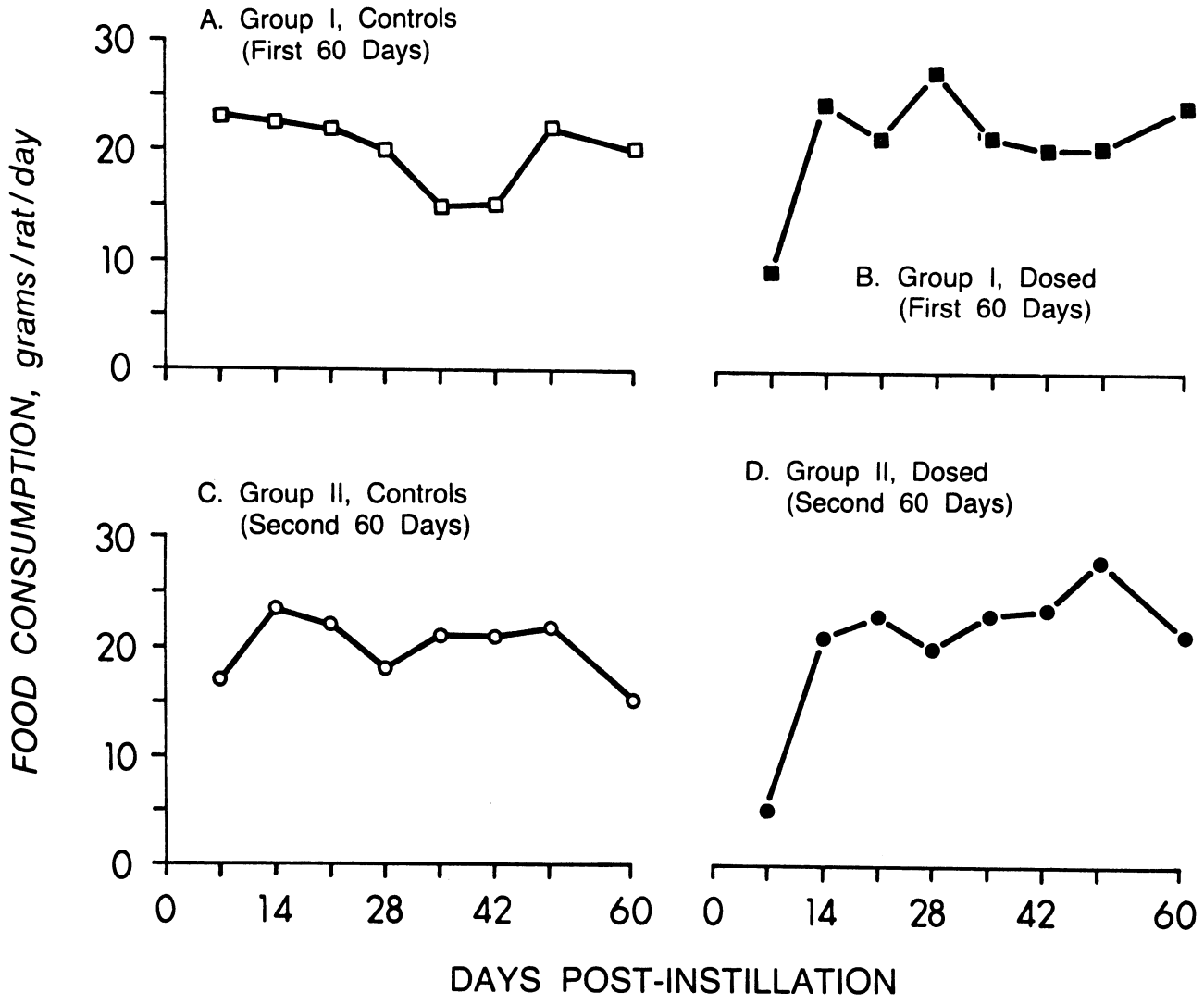

Fig. 70 Daily food consumption during days 7-60 post-instillation of one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

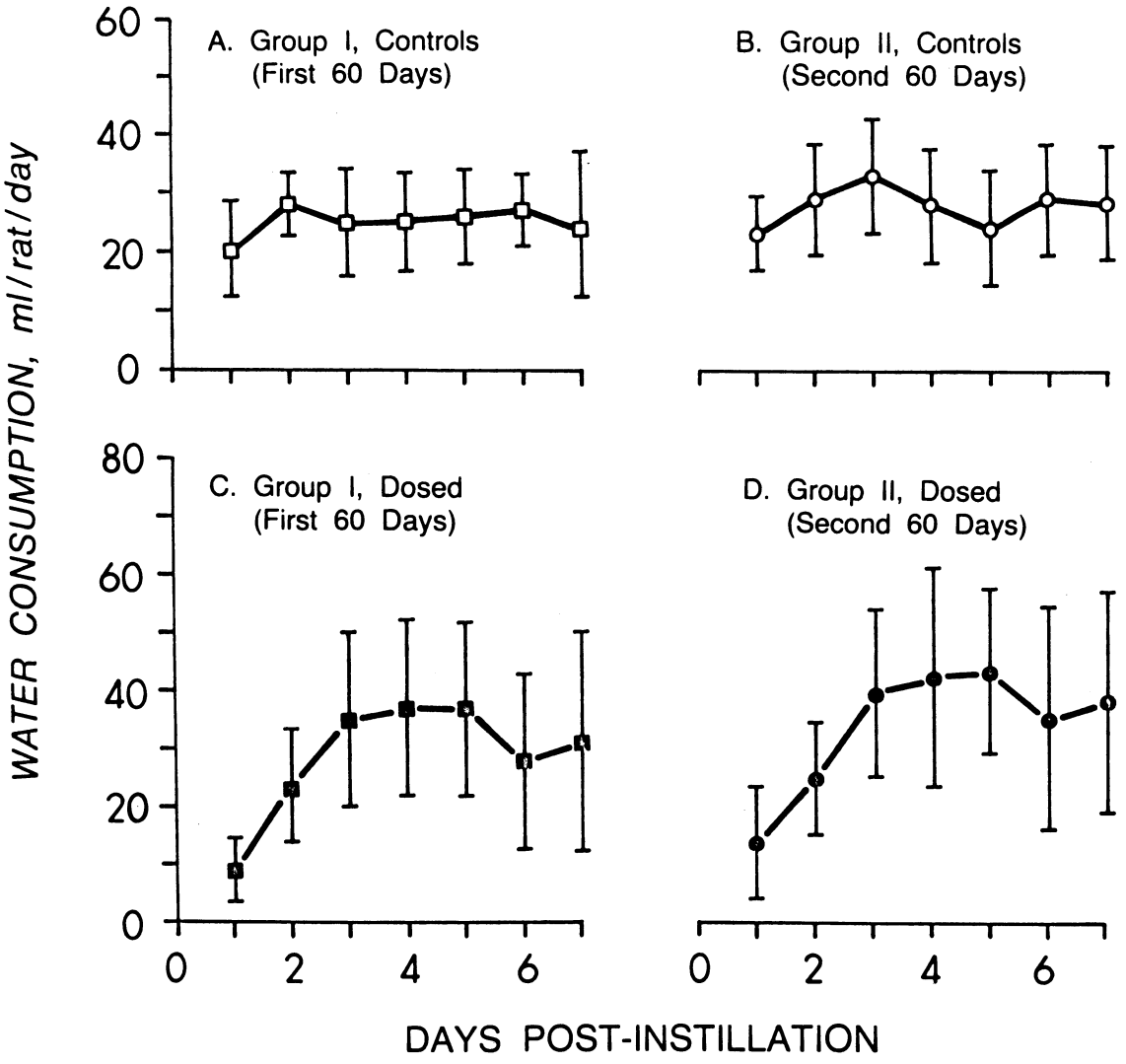

Fig. 71 Daily water consumption during days 1-7 post-instillation of one (control, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

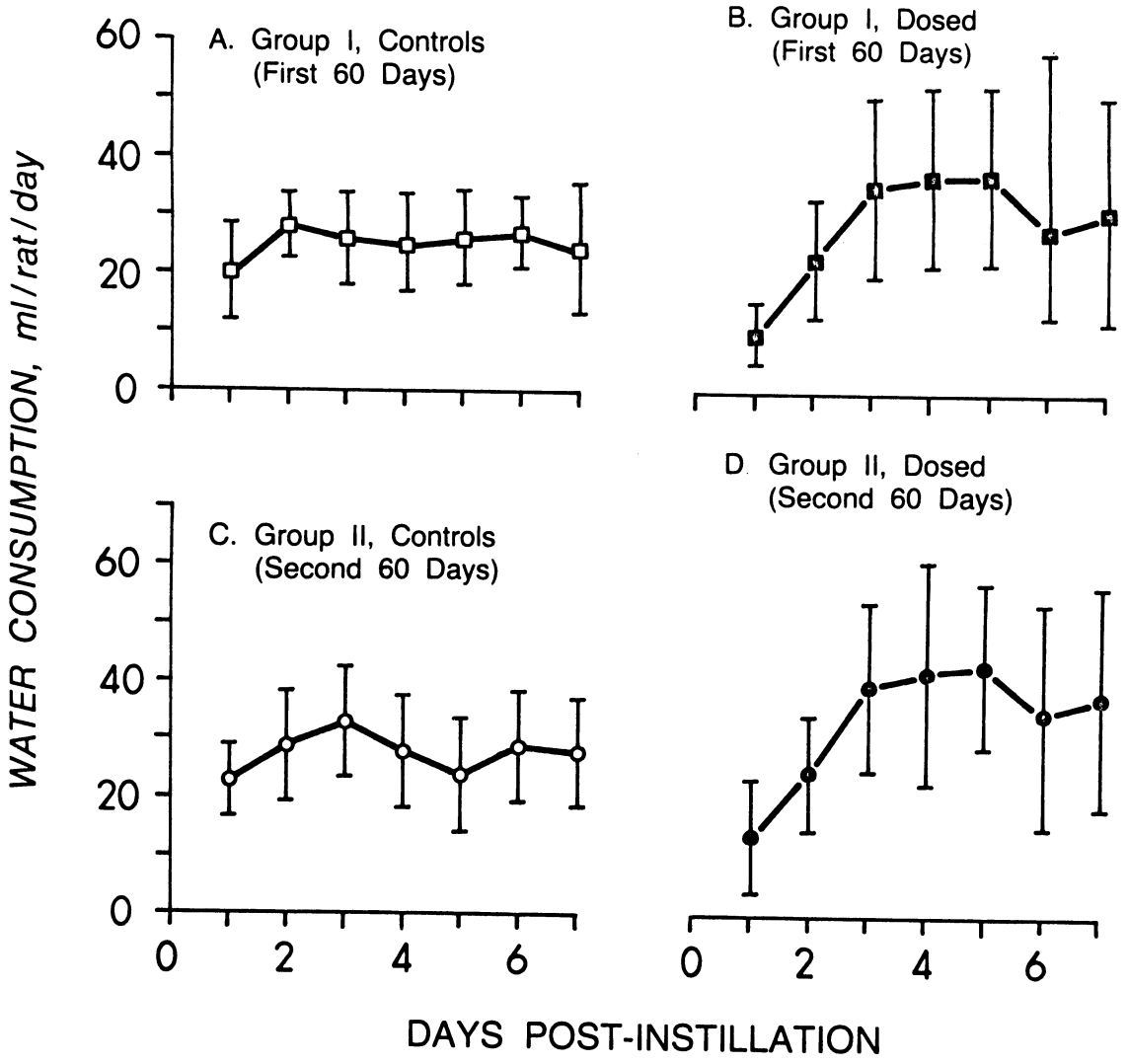

Fig. 72 Daily water consumption during days 1-7 post-instillation of one (control, □ ; dosed, ◘) or two (controls, O ; dosed, ●) instillations of 2 mg UO_2F_2/kg.

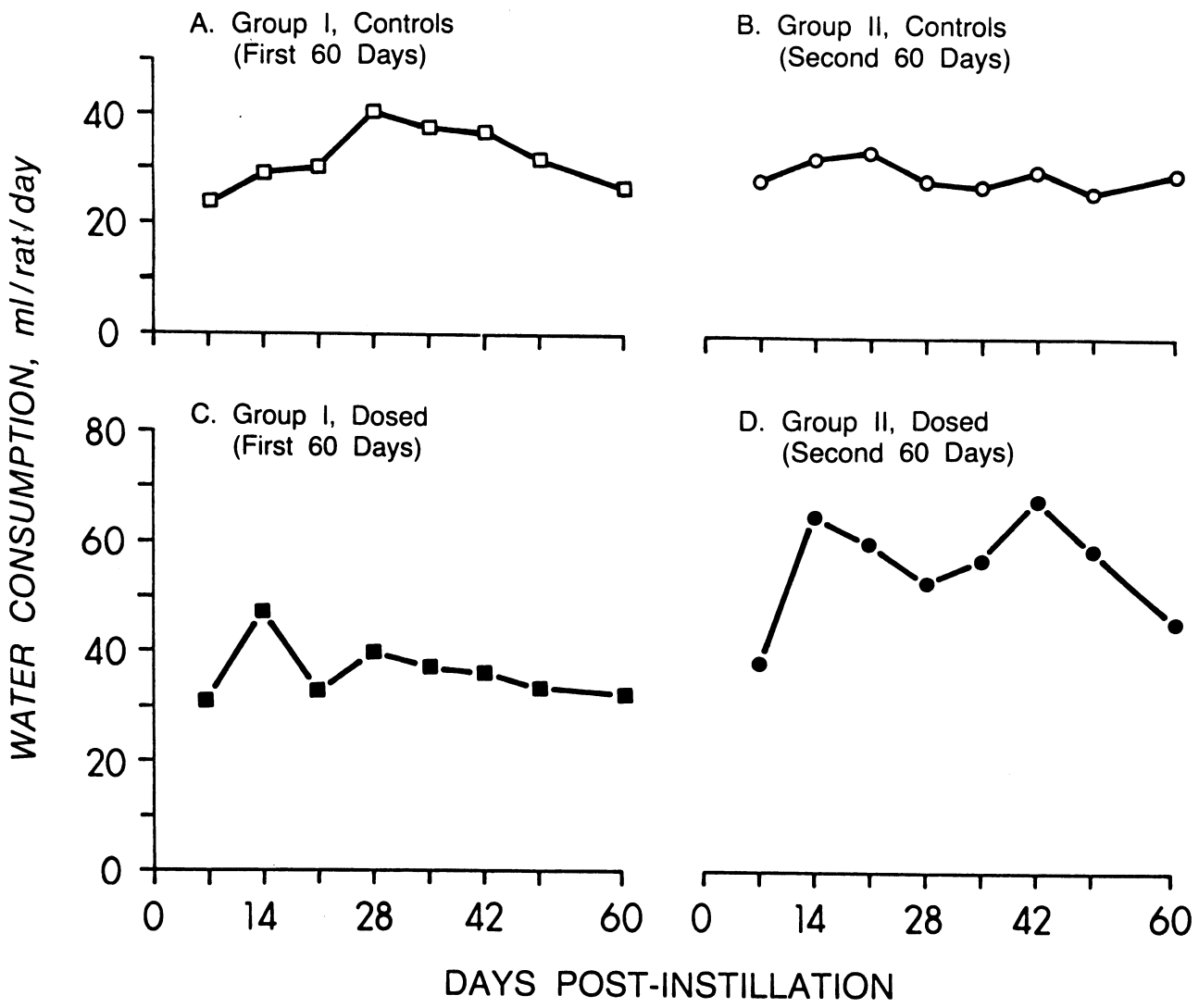

Fig. 73 Daily water consumption during days 7-60 post-instillation of one (control, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

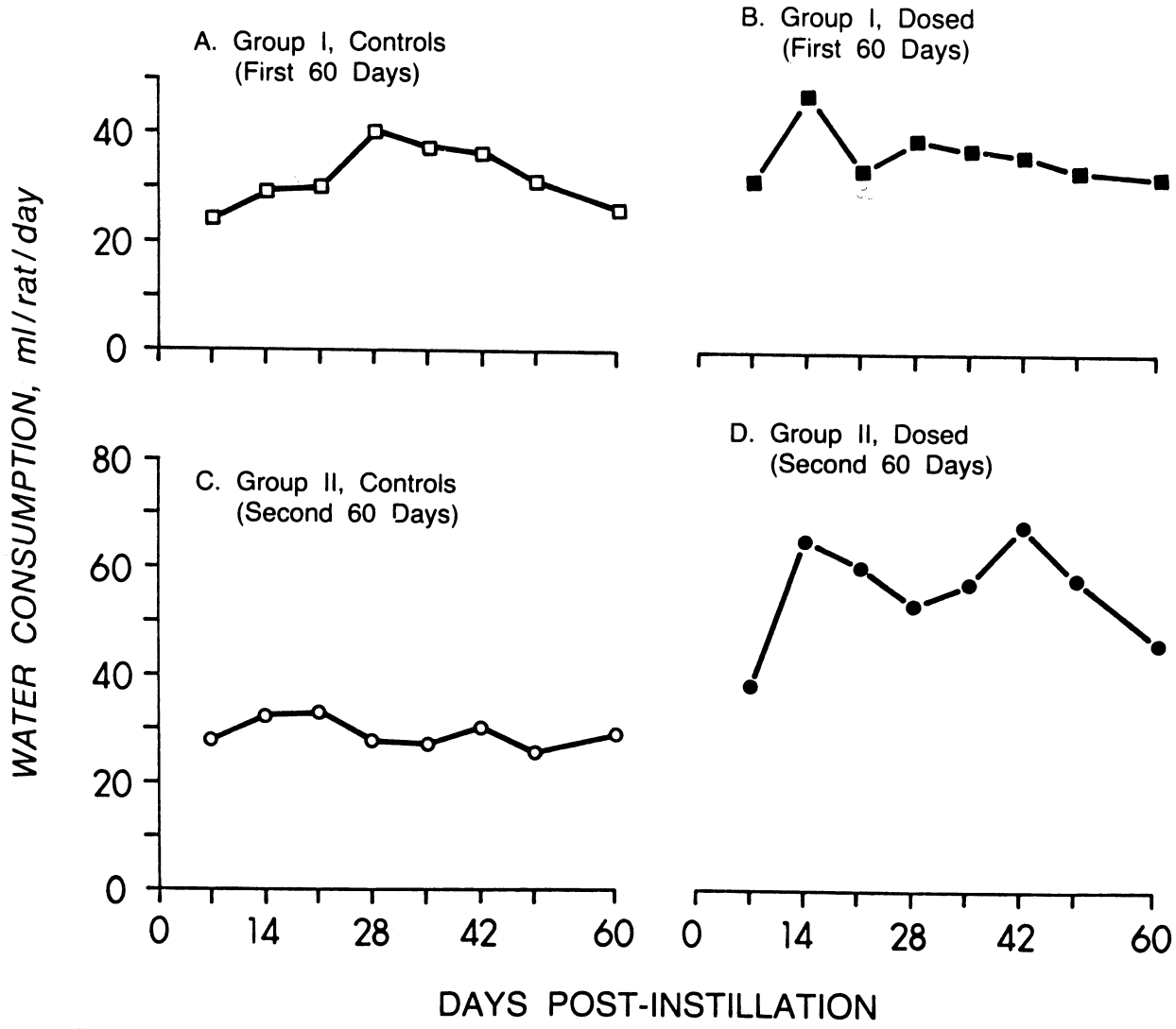

Fig. 74 Daily water consumption during days 7-60 post-instillation of one (control, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

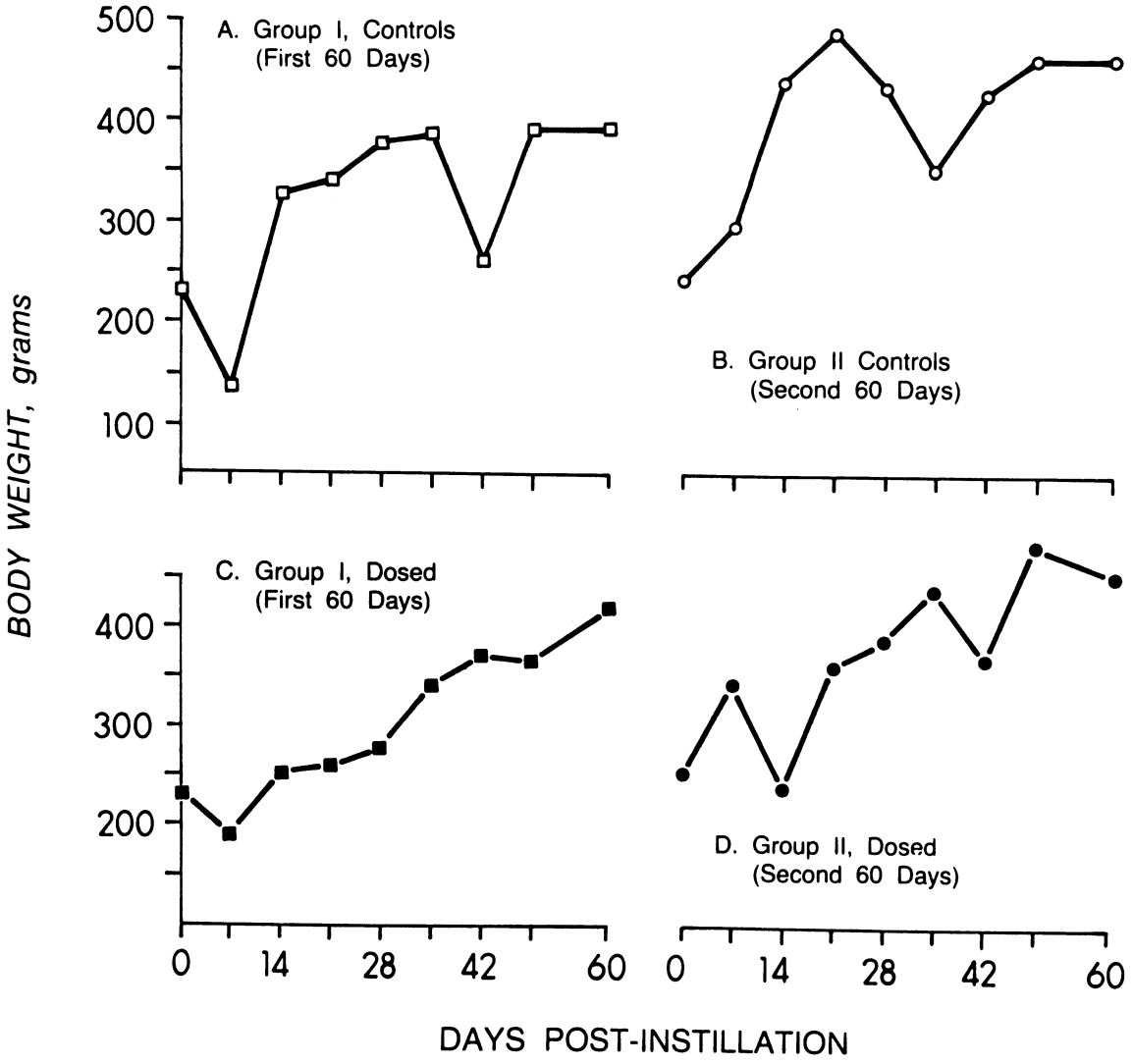

Fig. 75 Body weights of rats after one (control, □; dosed, ■) or two (control, ○; dosed, ●) instillations of 2 mg UO_2F_2.

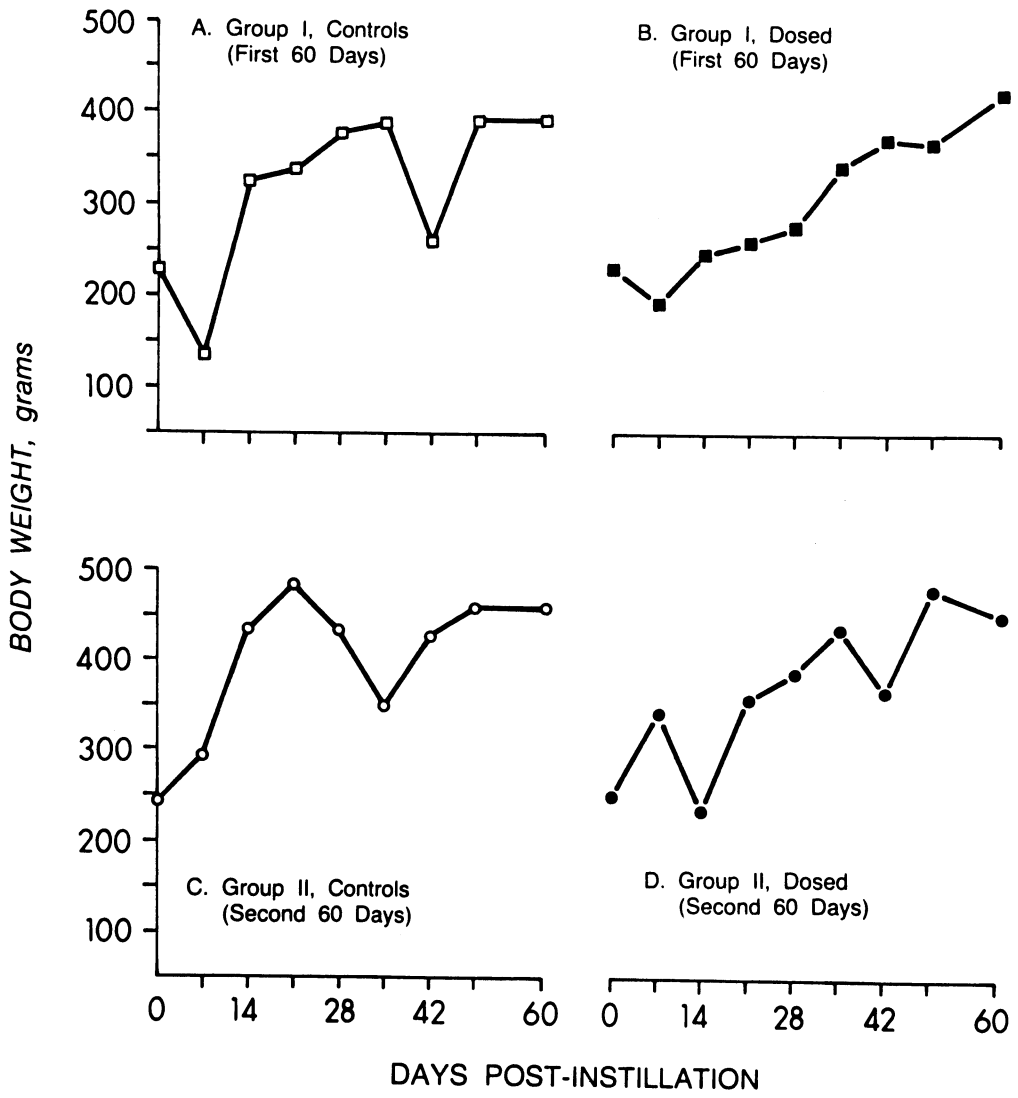

Fig. 76 Body weights of rats after one (control, □ ; dosed, ■) or two (control, O ; dosed, ●) instillations of 2 mg UO_2F_2.

than those of Group I, as would be expected inasmuch as these animals are 60 days older than those of Group I. Dosed animals in Groups I and II increased in weight over the 60 days, and again, the older Group II animals tended to be heavier than those of Group I. The pattern of response of the twice dosed rats is not considered to be significantly different from that of the single dosed animals (Fig. 75D vs C). However, neither the single nor double dosed animals grew as well as their respective controls (Figure 76B and 6A; 76D and C), a reflection perhaps of the lesser amounts of food ingested by the dosed rats during the first week after treatment (Figure 68B vs 68A and 68D vs 68C).

e) Kidney weight: Weights of the kidneys in grams and as organ weight/body weight ratio are shown in Figures 77 and 78. Organ weights of the control animals receiving a single instillation of the buffer did not differ appreciably from those controls receiving two instillations (Figure 77A and B), nor did there appear to be any greater effect of two instillations of UO_2F_2 compared to one instillation of the compound (Figure 77C and D). Comparisons of Figure 77E with 77F, and of Figure 77G and 77H suggests the kidney weights of the treated rats may generally be slightly less than those of their respective controls, but the differences are not pronounced.

Figure 78 shows the organ weight expressed as a fraction of the body weight. The ratio did not change with time for the control rats of either Group I or Group II, and was not different in the two groups (Figure 78A and B). Comparison of Figures 78C and D suggests that for both groups the ratio was higher in the first half of the post-instillation period. This effect seems to be more pronounced in the once dosed rats than in the twice dosed animals. Figure 78E and F and Figure 78G and H show that the early organ weight/body weight ratios in either the once or twice dosed animals exceeded the ratio in the corresponding control group. This relationship is also more prominent in the once dosed animals than in the twice dosed rats. Since the kidney weights did not increase, the higher early ratios then reflect an initially lower body weight, as was shown to be the case in Figure 76.

f) Combined exposure to UO_2F_2 and HF: As described earlier (Morrow et al., 1980) rats were given intratracheal instillations of UO_2F_2 or HF alone or in combination in dosages corresponding to the complete hydrolysis of 0.27 mg UF_6/kg^{-1}. The dosages of HF and of UO_2F_2 were 60 μg/kg and 0.23 mg/kg^{-1}, respectively. Responses to the UO_2F_2 alone measured during the next 10 days showed transient increases in urinary excretion of protein and glucose, urine volume, and plasma urea N, but all criteria were normal by 10 days post-instillation. Instillation of HF alone did not appreciably alter these measurements. When both compounds were administered together, the effects seen by UO_2F_2 alone were seen again, but they were not enhanced by the added presence of HF. In this experiment blood fluoride from both sources in combination can be calculated to be approximately 1 μg/F/ml. As indicated earlier,

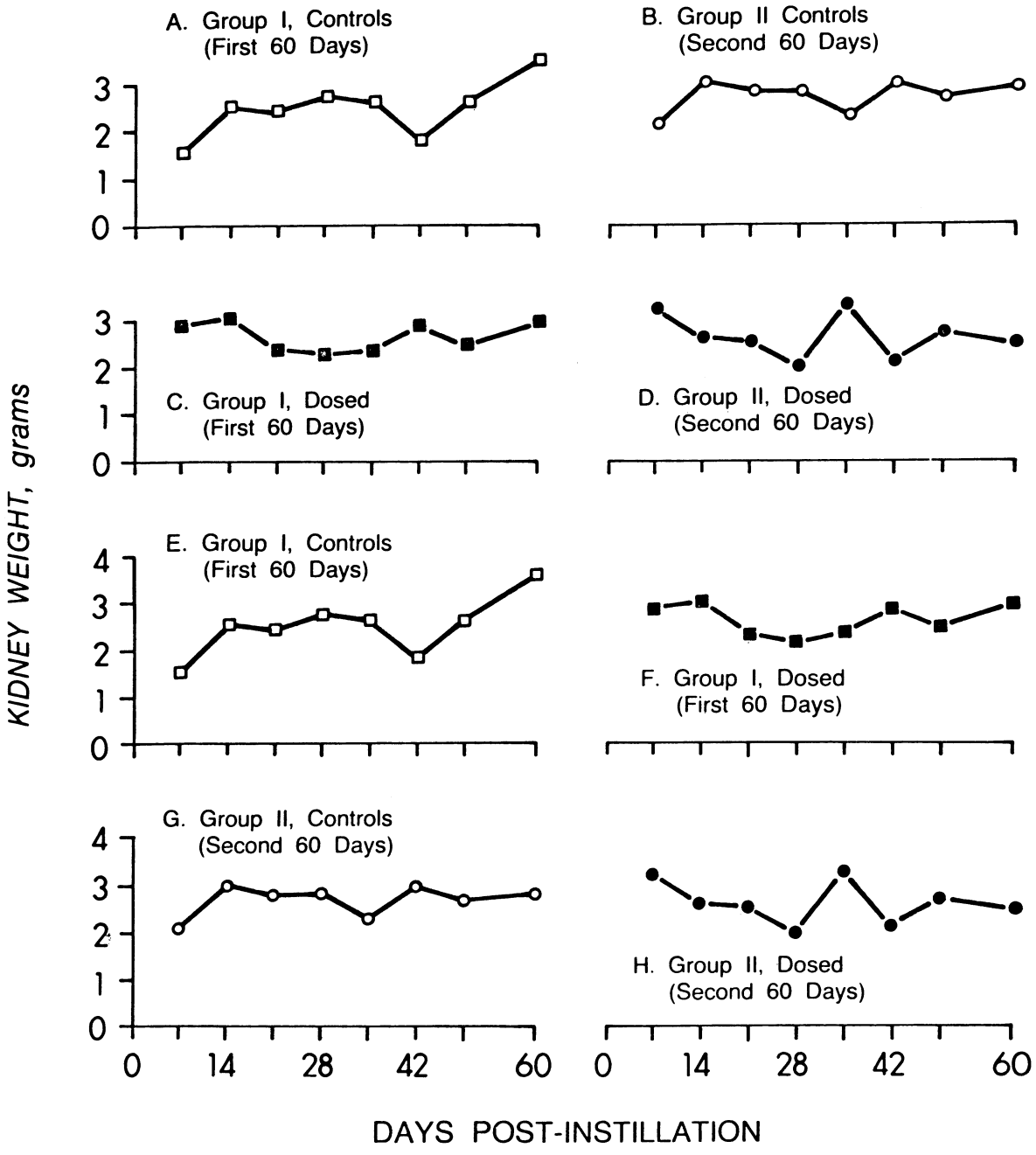

Fig. 77 Kidney weight during days 7-60 post-instillation of one (control, □; dosed, ■) or two (controls, ○; dosed, ●) instillation of 2 mg UO_2F_2/kg.

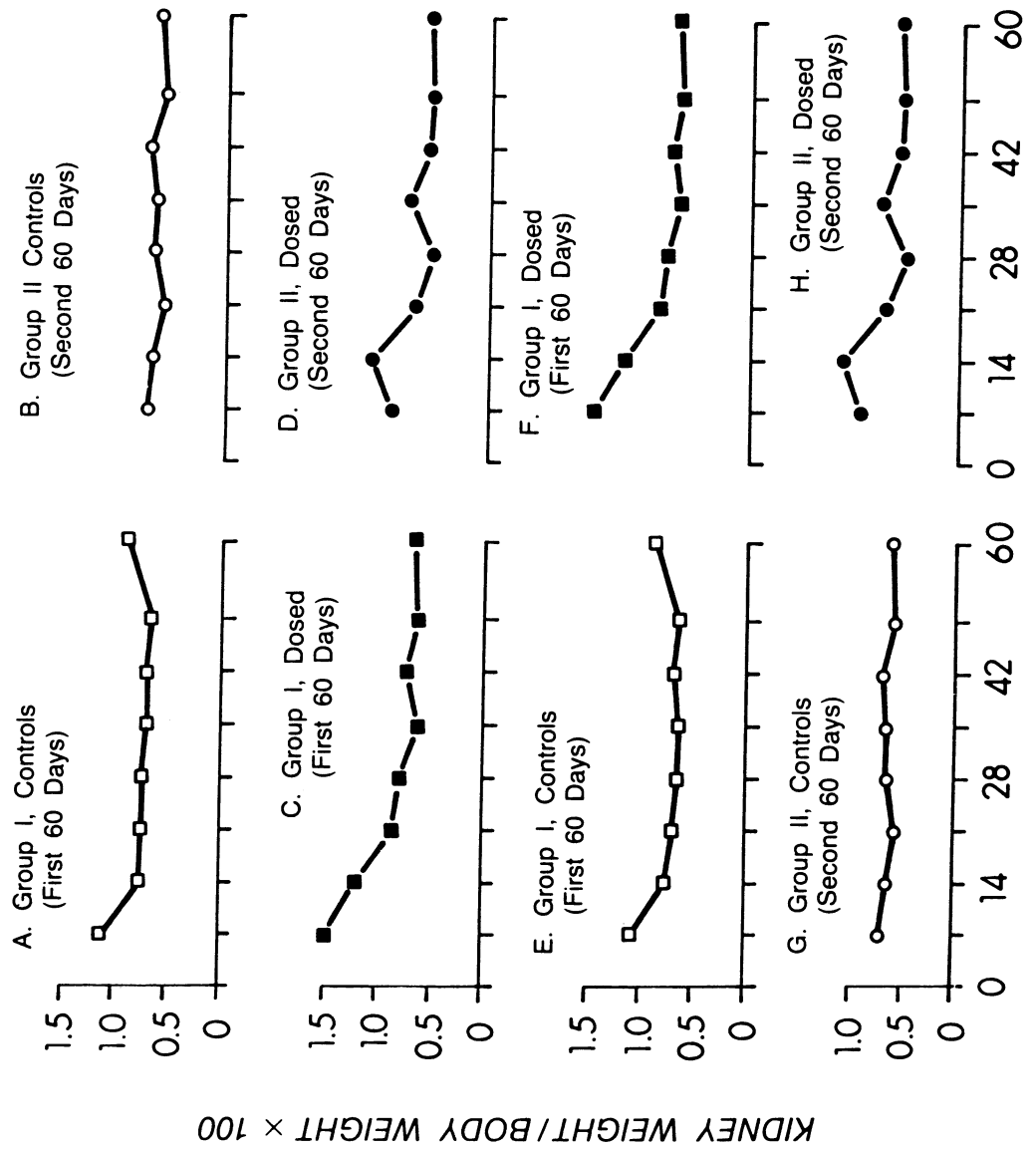

Fig. 78 Kidney weight as a fraction of body weight after one (controls, □; dosed, ■) or two (controls, ○; dosed, ●) instillations of 2 mg UO_2F_2/kg.

concentrations of this magnitude or greater may be expected to have a diuretic effect. Apparently however, the dosage of 60 μg HF kg^{-1} was not sufficient to affect the degree of diuresis produced by the dosage of UO_2F_2 alone.

VII. DISCUSSION

The chemistry of hexavalent uranium in the kidney is described in the uranium monograph (Dounce, 1949). It is useful to visualize the excretion of uranium in terms of three phases: 1) filtration of a U^{+6}-bicarbonate complex by the glomerulus; 2) in the proximal convoluted tubule, concentration of the complex by water resorption, acidification of the filtrate by resorption of base and bicarbonate, breakdown of the bicarbonate complex, and the subsequent complexing of U^{+6} with either tubular protein (tissue fixation) or anionic components, e.g., organic acids and phosphate, which leads to excretion; and 3) excretion of the soluble complexes via Henle's loop and distal tubular segments. Although the model predicts fixation of U^{+6} only in the proximal tubule, the histopathology and biochemical indices of renal injury augur for more generalized sites of injury.

The study by Eisner, et al. (1968) also points out features of uranyl ion toxicity in the kidney. Using dogs, they demonstrated that in uranium-treated kidneys, renal plasma flow is reduced compared to normal; however, this is not sufficient to explain the oliguria seen in acute uranium poisoning. They raise the possibility that the reduction in inulin clearance (glomerular filtration rate index) may not be entirely due to reduced glomerular filtration. Evidence for a bidirectional movement of inulin into additional renal compartments (intracellular or intraluminal or both) was found, that is, the apparent volume of renal inulin distribution was increased by uranium.

A recent study by Nizet (1981) of intravenous uranyl nitrate in dogs demonstrated a prompt reduction in glomerular filtration rate without a change in urinary output. This indicated that the reduction in glomerular filtration counterbalanced the reduction in tubular reabsorption of fluid, the latter being a manifestation of the inhibition of sodium transport by the uranyl ion. The reduced glomerular filtration rate is not fully understood but Nizet offers evidence that this tubuloglomerular feedback is mainly due to an increase in proximal intratubular hydrostatic pressure (PTP). Others have not seen an increased PTP after uranyl nitrate but their work pertains to 6 to 48 hrs post injection, after the initial polyuria and natriuresis and while the dogs were hydropenic, so the studies are probably not comparable.

The use of biochemical indicators of renal injury was relatively extensive in this study, but it is evident that much more information is needed before a decisive answer can be given to the question: what is the best way to detect and follow uranium-induced kidney injury? In our experience, urinary glucose stands out. We believe it is more useful than urinary protein, consequently we recommend its consideration in monitoring and for individual assessments. However, we feel that there may be value in other indicators or combinations thereof, and certainly more must be learned about the nephrotoxic mechanisms of UO_2^{++} which appear to

be multiple and possess different susceptibilities, temporal relationships, and repair patterns (See Research Recommendations).

Evaluation of renal tubular function by analyzing the urine for specific proteins, enzymes or metabolites seems to offer excellent promise for finding sensitive indicators of U-induced nephrotoxicity. Since large amounts of these substances are filtered and resorbed daily, small decreases in tubular function can result in large changes in excretion. N-acetyl-β-glucosaminidase (NAG) is an enzyme that occurs in high concentrations in lysosomes of renal proximal tubules. The excretion of N-acetyl-β-glucosaminidase (NAG) was examined in these studies as a marker of renal hydrolase release. NAG appears to be somewhat more responsive than urinary protein. The bimodal increase in NAG excretion (See Figure 22 and Table 12) seems to be a reproducible finding which may reflect an extension of necrosis from one tubular region to another.

Our studies of uranium-induced tolerance failed to demonstrate an increased citrate excretion as was reported by Haven and Randall (1949). In the 30 years since the original citrate measurements, assay methods have changed; this may be responsible for the different findings in the two studies. We examined the possible effect of UO_2^{++} on citrate-lyase and malic dehydrogenase and on the possible formation of uranyl-citrate complexes in the urine, without finding evidence for either. Thus, while we remain uncertain about the basis for the disagreement between the new and old studies, we are nevertheless confident that the earlier work is in error.

The recent work of Stevens, et al. (1980) on the metabolism of ^{233}U-labelled uranyl citrate after intravenous administration in beagles is cited several times in this report. This was an unusual study of hexavalent uranium, principally because of its approximately two year duration, thereby providing the best experimental basis available for determining the more protracted retention times of uranium in kidney and bone. Unfortunately, their 726 day retention data were compiled from the serial sacrifice of only 7 beagle dogs. Not unexpectedly, the computed half-times are very sensitive to the reported value for a single dog, especially in the case of the renal retention; for example, the biological half-time decreases from 79.5 days to about 53 days if the last dog sacrificed is eliminated. On the same basis the mean skeletal retention half-time for uranium would increase from 883 days to more than 2000 days. Nonetheless, their findings are particularly important, for they clearly indicate that there is significant long-term uranium retention in these tissues governing approximately 15 and 7.7 percent of the administered dose, respectively. Additional retention functions are given by Stevens et al. for soft tissues other than kidney, averaging about 0.0036 day^{-1} for the lung, for example, but constituting only 0.02 percent of the dose. All such functions are subject to the same uncertainties regarding the kinetic analyses, but again, there is little doubt that a small, long-term retention component appears to be associated with most

soft tissues. The basis for this protracted retention is unknown, but it is conceivable that turnover of skeletal uranium may contribute. On the same point, in a summary of the human retention data for uranium by Adams and Spoor (1974), kinetic analyses are given that either utilize a two-term exponential function or a simple power function. These analyses attest to the fact that such retention data are amenable to a number of mathematical treatments and no unique kinetic description applies. Secondly, they also attest to the presence of long-term retention components for uranium in human tissues.

Walinder, et al. (1967) studied uranyl acetate and U_3O_8 in several species and concluded that the renal retention of uranium is greatest initially in the cortex. The subsequent disappearance of uranium from the kidney is slower for the cortex than for the medulla, but both are faster than for other soft tissues, e.g., liver and spleen. This general observation was confirmed by Stevens, et al. (1980) with their 79.5 day T 1/2 for kidney and 170-190 day half times for several other soft tissues in the dog. Walinder, et al. concluded that several months after a single intake of uranyl acetate, the kidney level will be less than the spleen: for this and other reasons, they regard the spleen and bone depots as a better basis for limiting uranium exposure than the renal level. This argument can only have validity when the radioactive dose from uranium is more important than its chemical toxicity. This viewpoint, therefore, cannot apply to natural uranium compounds.

The prolonged soft tissue retention of uranium (exclusive of the kidneys) determined in the UO_2F_2 studies and reported by others, has not been evaluated in relation to steady state kinetics, under chronic exposure conditions, or where some grade of enriched uranium is used. In all the cases cited, the fractions of a given intake which remain in the extrarenal soft tissues, and exhibit long retention times, are always less than 0.05 and usually less than 0.01. Irrespective of the potential health physics implications, this persistence of uranium suggests there may be some limited binding sites in many tissues possessing a high affinity for uranium. We examined in two pilot experiments the possibility of metallothionein involvement and also examined the canine kidney for evidence of an inducible ligand, since we had found the kidneys of multiple-dosed animals retained a greater fraction of the absorbed dose. Both efforts produced negative results.

The NRC action level of 30 $\mu g\ U\ l^{-1}$ urine, like the renal threshold concentration of 3 $\mu g\ U\ g^{-1}$, is obviously both a dose and time dependent response. Hence the appearance of 316 $\mu g\ U\ l^{-1}$ in the urine of subject 2 (Rochester Study), who received 6.3 $\mu g\ U\ kg^{-1}$ intravenously during the first 9 hours post injection (1143 ml containing 361 μg U), is strikingly different from 22-46 hour urine which averaged 3.7 $\mu g\ U\ l^{-1}$. This injected dose was 1/10 of the lowest dose producing positive urinary changes in that study (Bassett et al., 1948). This example, typical of the data

obtained in naive dogs at the lowest intravenous and inhalation doses, fits well with the lowest dose, intravenous studies in man (Bassett, et al., 1948). Therefore, the first 24 hour urinary uranium excretion can be expressed as 63 percent of the absorbed dose and the subsequent excretion rate $Yu = 0.29t^{-1.5}$. Together these indicate a total urinary elimination of 92 percent of the absorbed uranium is expected.

The excretion rate of urinary uranium after UO_2F_2 exposure was invariably lower in rats than in dogs. Rats after UO_2F_2 inhalation provide 0.48 and $Yu = 0.18t^{-0.85}$, respectively, as the daily excretion rates when the data are presented in the ICRP form. The fact that rats were generally exposed to higher UO_2F_2 doses than the dogs may be involved in this apparent specie distinction. The inhalation and intravenous studies in rats and dogs both showed reduced U elimination in the urine with the higher doses and from previously-exposed animals. This was not the finding in the intratracheal studies at 2 mg UO_2F_2 kg^{-1} instilled in a buffered aqueous solution. In these studies, the rats appeared to excrete more uranium after the second dose than with the first 2 mg kg^{-1} dose. The reason for this difference is not understood.

Once again, as reported in NUREG/CR-1045 (Morrow et al., 1980), rats demonstrated high fecal excretion of U, that is 47 to 67 percent of their inhaled dose upon exposure, this time to hydrolyzed UF_6 (UO_2F_2 + HF). The particle size of the UO_2F_2 aerosols ranged from 0.88 to 1.80 μm, MMAD (σ_g from 1.61 to 1.92) and showed a direct relationship to the percent of the inhaled dose of U excreted in the feces, that is, the smaller the aerodynamic particle size, the less U excreted in the feces. The inhalation of HF at levels produced in these experiments did not appear to modify appreciably the fecal excretion of uranium.

UO_2F_2 is extremely soluble in water and we have presented data that shows it is rapidly absorbed through the respiratory tract. It therefore seems odd that the rat could excrete such relatively large quantities of this inhaled material through the gastrointestinal tract (GIT) before absorption occurred. From the large amounts of U found in the feces, GIT (see Figures 15 and 18) and upper respiratory tract (nasopharyngeal region, Figure 16), we deduce that the rat, an obligatory nose breather, traps UO_2F_2 particles in the nasopharyngeal region quite efficiently and these particles are promptly swallowed and passed through the GIT. Since U^{+6} compounds are poorly absorbed from the GIT (1 percent absorption - see Table 5, Experiment No. 10), the UO_2F_2 in this site is almost quantitatively excreted in the feces.

The intratracheal studies described herein permit a comparison of the toxicological profiles induced in rats receiving the same dosage of UO_2F_2 administered on two successive occasions spaced 60 days apart. The dosage used is associated with a renal burden of 3 μg U g^{-1} of tissue. The characteristic features of uranium poisoning were engendered after each dose. Food intake was initially

depressed; treated animals did not grow as well as controls; water intake and volume of urine excreted increased; urinary excretion of protein and glucose and plasma urea N were temporarily elevated; structural damage in the renal tubules was evident histologically. A comparison of responses after the two doses shows some significant differences, however. Mortality was less after the second dose, and the deaths were delayed. Urinary glucose increased to maximal concentrations on day 4 after one or two doses, but the peak concentration was less after the second dose and required twice as long to return to normal. Maximal plasma urea N concentrations also were less after the second dose, and required twice as long, or more, to return to normal again. A persistent diuresis was caused by one or two doses, but was more pronounced after the second dose. The volume of water ingested was persistently elevated after two but not after one dose. Renal tubular degeneration was seen after one or two doses, and histopathology was still evident 60 days after either dose; however, the damage was more widespread in the kidneys of the twice-dosed animals. Total fecal excretion of uranium was greater in the twice-dosed animals. As already mentioned, effects on the urinary excretion of uranium are hard to judge inasmuch as excretion via this route was inexplicably low after the first dose. Mobilization of uranium from the kidneys was approximately halved after the second dosing. These findings demonstrate that the biochemical effects of the first dose of uranium had returned to normal during the course of the next 60 days, but that morphological effects on the kidney had not, nor had the associated increase in urine volume been normalized. A deficit in renal capability at the time of the second dose was revealed by the longer times required for plasma urea N and urinary protein and glucose to return to normal, by the greater extent of histological damage which was still apparent 60 days after the second dose, and by the greater than normal daily urine volume.

The lesser mortality in the twice-dosed animals and the increase in time to death and lower peak responses in some biochemical indicators all suggest that a "tolerance" to uranium had been induced in the once-dosed animals. However, the concept of tolerance as developed by Haven (1949) was based only on the ability of the tolerant rat to survive a lethal challenge dose of uranium. The presence of regenerated "atypical" renal tubular epithelium and large amounts of citrate in the urine were considered essential to the development of tolerance, and no investigations were made into the long-term renal capabilities of the tolerant rat. In view of the more extensive findings described here, it seems unwise to consider that a long-term protection has been established. The longer time required for the biochemical indicators to return to normal, the slower clearance of uranium from the renal tissue, and the presence of atypical repaired tubular epithelium and lesser functional renal parenchyma all indicated an impaired renal capability which over a period of time may over balance any short term gains in lesser biochemical response and mortality.

Comparisons of the toxicity of UO_2F_2 with and without HF present were made in rat (experiments 4 and 11) and dog (experiments HF-2 and I-15) inhalation studies, with similar findings, namely, that some evidence of slightly greater renal impairment, e.g., proteinuria, glucosuria and polyuria, resulted from the combined UO_2F_2-HF exposure than from a comparable dose of UO_2F_2 alone. No other differences in distribution, excretion and effects were detected. The intratracheal administration of UO_2F_2 into rats provided no distinctions between UO_2F_2, with and without HF present in the aqueous instillation solution, in the one experiment intended to test this.

In most of the inhalation experiments involving hydrolyzed UF_6, we measured precisely the UO_2F_2 aerosol concentration and then calculated the corresponding yield of HF by using the chemical equation: $UF_6 + 2\ H_2O \rightarrow UO_2F_2 + 4\ HF$. These theoretical values for HF were tabulated for each rat inhalation experiment (See Results, Table 6).

As our studies progressed, we attempted to verify the actual HF concentrations in the exposure chamber atmosphere by collecting samples with two in-line miniature bubblers each containing 5 ml of TISAB reagent. These bubblers were placed immediately behind the filter paper dust sampler (used to collect UO_2F_2 aerosol) in the air sampling train whose flowrate of 250 cc min^{-1} was established and maintained by a critical orifice and vacuum source.

All parts of the sampling train were essentially plastic i.e., polyethylene, polypropylene, polycarbonate or Teflon and were dry at the beginning of the sampling procedure. Membrane filters (13 mm dia.) used to collect the UO_2F_2 aerosol were either standard Millipores (mixed esters of cellulose) or Nuclepores (polycarbonate) of 0.8 μm pore size.

Our findings were somewhat puzzling. Although very little HF penetrated to the second bubbler, it was found in the tubing before the first bubbler, in the first bubbler itself and on the membrane filter containing UO_2F_2. The total HF recovered was variable and never exceeded 50 percent of the calculated value. From these results we concluded that a significant amount of the HF produced upon the hydrolysis of UF_6 reacts with or adsorbs to surfaces it contacts. Therefore, the exposure chamber air concentration of HF most likely was lower than calculated and losses in the sampling train reduced the HF concentrations analyzed even more. Problems with the analyses of co-existent gas and aerosol phases are not unique to this study nor to UF_6 decomposition (NIOSH 1976).

Our best estimate at this time would be that for the exposures reported here, the actual concentration of HF which the animals breathed was on the order of 50 percent of the theoretical values reported in Table 6.

Although under the conditions of our experimental exposures, a slightly additive nephrotoxic effect was noted, effects of fluoride intoxication *per se* were not expected nor detected. Similarly, under the conditions of health physics and industrial hygiene control normally prevailing in UF_6 operations, no significant effects of fluoride exposure are to be expected. Blood fluoride determinations were not made in these animals, but assuming that all of the fluoride present in the UO_2F_2 administered intratracheally is available as ionic fluoride in the circulating blood, one can calculate that maximal blood fluoride concentrations of the order of 3 μg F/ml may have been present briefly in these rats. According to Cousins and Mazze (1973) blood fluoride greater that 1 μg/ml can lead to polyuria in man. There is some possibility, therefore, that fluoride released from the absorbed UO_2F_2 may have contributed in part to the diuresis seen. Therefore, measurements of blood fluoride in the immediate post-exposure evaluation of victims of a large accidental release of UF_6 may be of value in assessing their renal status.

VIII. RESEARCH RECOMMENDATIONS

Many interesting and potentially important areas of investigation were necessarily curtailed or omitted from the 2-year study because of time constraints and priorities. Among these are the following:

(1) Determination of the gas and particulate phase composition when UF_6 is spontaneously hydrolyzed in air. Of special interest are the possible associations of HF with the hydrated UO_2F_2 aerosol and the need for a mass balance approach wherein the sampling methods are both quantitative and artifact-free.

(2) Experimental production of 3 μg and 0.3 μg U g^{-1} kidney levels in laboratory animals by relatively continuous exposures, thereby achieving the steady state levels without ever exceeding these respective renal concentrations. Animals in the steady state conditions could then be evaluated as to U retention after exposure cessation, urinary U output while exposure-maintained and after exposure cessation, by urinary biochemistries in both situations, and by histological examinations.

(3) Additional renal function methods should be assessed in both naive and previously-exposed animals over a dose range which encompasses the acute production of the minimal renal injury level. Catalase, β-microglobulin, leucine amino peptidase and LDH are among the indicators which should be included in the needed, modern evaluation of uranium toxicity. Refinements of existing approaches are also important. For example, although it is known that aminoaciduria is a sensitive index of uranium injury, several stereospecific absorption systems have been demonstrated for different classes of amino acids, consequently it is possible that UO_2^{++} has differential effects on these systems which could be exploited by analyses for specific amino acids.

(4) The development of "tolerance" to uranium-induced renal injury was judged principally in the 1940 studies by reduced mortality. The results of this 2-year study indicate that in sub-lethal terms, which are the more relevant, the induction and persistence of any specific protective effect is often complex and time-related. The overall picture is clearly far more complicated than the early studies implied, consequently a systematic dose-time evaluation should be undertaken incorporating functional assessments directed at both glomerular and tubular areas. Also of potential interest are comparisons of UO_2^{++} with mercuric ion and aspirin-induced renal damage for which tolerance is also known to develop.

(5) A related area of needed research concerns the sites and mechanisms of renal damage from uranyl ion. Studies were cited in this final report on efforts to determine the nature of reduced glomerular filtration rate after uranyl ion administration. Uranyl ion interactions with tissues are believed to be relatively non-specific: reactive ligands include phosphoryl and carboxyl groups. The initial reaction sites are limited presumably to the cell surface but UO_2^{+2} may penetrate into injured cells.

The transport of amino acids and glucose are coupled to Na^+ transport in the proximal tubule, i.e., a Na^+-K^+ activated ATPase located at the peritubular membrane surface. In our study, uranyl fluoride inhibition of glucose required three to four days before becoming maximal. Recently Nechay et al. (1980) showed that the kidney ATPase is very sensitive to UO_2^{++}, so it is possible that glucose absorption is not impaired until the driving force for transport located at the basolateral tubular membrane is inhibited meaning the inhibition is not directly related to carrier-mediated systems located on the luminal surface. It is also interesting that the ascending limb of the loop of Henle has been found to possess 4-5 X the Na^+K^+ dependent ATPase activity of the proximal tubule, while other studies have suggested that U^{+6} may act at the peritubular membrane (Nomiyama and Foulkes, 1968; Foulkes, 1971).

It is clear from these research findings and from the evidence for more widespread damage to the nephron than to the distal-third of the proximal tubule, that our views of uranium nephrotoxicity are both inaccurate and incomplete.

(6) Another related area for research concerns the bases for the soft tissue retention of uranium. Although our pilot studies were unproductive, many approaches to the problem remain which could logically be associated with a chronic intake study such as with suggestion (2) in these Recommendations. It would be particularly interesting to perfuse temporarily an organ, e.g., kidney, with a homologous blood circuit through which the organ could be dosed with UO_2^{++} without a concomitant bone uptake. The role of bone-associated uranium in maintaining a circulatory (and soft tissue level) could be assessed, thereby, and an improved basis for investigating soft tissue retention of uranium would be provided.

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16. ABSTRACT *(200 words or less)*

This final report summarizes the experimental studies undertaken in rats and dogs in order to ascertain whether or not the NRC bioassay program has adequate biological bases for quantifying and evaluating uranium hexafluoride exposures. Animals were administered the spontaneous hydrolysis products of uranium hexafluoride by inhalation exposures, intratracheal instillations and parenteral injections. Attention was given dose-effect relationships appropriate to the kidney, the unique site of subacute toxicity; to the rates of uranium excretion; and to uranium retention in renal tissue. These criteria were examined in both naive and multiply-exposed animals. Findings of the studies: partly substantiate the ICRP excretion model for hexavalent uranium; generally provide a lower renal injury threshold concentration than implicit in the MPC for natural uranium; indicate distinctions in response based on exposure history, e.g., uranium excretion rate; compare and evaluate various biochemical indices of renal injury; raise uncertainties about prevailing views of "reversible" renal injury, renal "tolerance" and possible hydrogen fluoride synergism with uranium effects; and reveal species differences in several areas, e.g., renal retention of uranium. While the study presents some complicating features to extant bioassay practice, it nevertheless supplies data supportive of the bioassay concept.

17. KEY WORDS AND DOCUMENT ANALYSIS

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uranium hexafluoride	UF_6
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