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Inhibition by Uranyl Nitrate of Adenosine Triphosphatases Derived from Animal and Human Tissues

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Inhibition by Uranyl Nitrate of Adenosine Triphosphatases Derived from Animal and Human Tissues. NECHAY, B. R., THOMPSON, J. D., AND SAUNDERS, J. P. (1980). *Toxicol. Appl. Pharmacol.* **53,** 410–419. Inhibition of adenosine triphosphatase (ATPase) by uranyl nitrate (UO_2^{2+} or U^{6+}) was studied in microsomal fractions and tissue homogenates of several organs and species. U^{6+} inhibited ouabain-sensitive ($Na^+ + K^+$-dependent) ATPase and ouabain-insensitive (Mg^{2+}-dependent) ATPase with I50 of 2×10^{-5} to 2×10^{-4} M. Higher concentrations of U^{6+} were required to inhibit the enzyme in homogenates than in microsomal fractions. Mg^{2+} ATPase was somewhat more sensitive to U^{6+} than was $Na^+ + K^+$ ATPase when data were corrected for protein content of enzyme preparations. The inhibition of $Na^+ + K^+$ ATPase, but not Mg^{2+} ATPase, was markedly antagonized by Na^+. This suggests that U^{6+} may inhibit $Na^+ + K^+$ ATPase at the Na^+ site on the enzyme, whereas ouabain inhibits at the K^+ site. ATP decreased and Mg^{2+} increased the inhibition of both enzymes. K^+ had no effect. The remaining studies were done with $Na^+ + K^+$ ATPase. Increasing pH enhanced inhibition. The enzyme was protected by bovine serum albumin and citric acid. Ascorbic acid increased inhibition possibly by reducing U^{6+} to U^{4+}, thus rendering the new ionic species reactive with sulfhydryl groups in addition to organic anions.

Radiotoxic health hazards from excessive exposure to uranium and its salts have been recognized for a long time. On the basis of extensive studies associated with the Manhattan Project, it was considered unlikely that an ordinary industrial exposure to uranium posed any significant chemotoxic threat. However, under experimental conditions, uranium is capable of producing renal damage by chemical action (Hodge *et al.*, 1973; Voegtlin and Hodge, 1949/1953).

In the body uranium is present in tetravalent (U^{4+}) or hexavalent (U^{6+}) forms, since its other oxidation states (U^{3+} and U^{5+}) are unstable under *in vivo* conditions. U^{6+} is the most stable form and it exists as the oxygenated cation UO_2^{2+}. Both U^{4+} and U^{6+} form complexes with anions such as HCO_3^-, $-COO^-$, and groups containing phosphate radicals. Unlike U^{6+}, U^{4+} has some affinity for –SH groups. No significant reduction of U^{6+} to U^{4+} is thought to occur in the mammalian body (Passow *et al.*, 1961; Voegtlin and Hodge, 1949/1953).

Uranyl nitrate ($UO_2(NO_3)_2$), a UO_2^{2+} or U^{6+} form, has been used in experimental animals to produce renal failure characterized by abnormal electrolyte excretion, proteinuria, glucosuria, aminoaciduria, tubular necrosis, and eventually anuria (Hodge *et al.*, 1973).

Diuresis with impaired urinary diluting and concentrating capacity is observed in uranium nephropathy (Bowman and Foulkes, 1970; Maher, 1976). A similar effect is observed when renal Na^+ and K^+-dependent adenosinetriphosphatase ($Na^+ + K^+$ ATPase) activity is inhibited with

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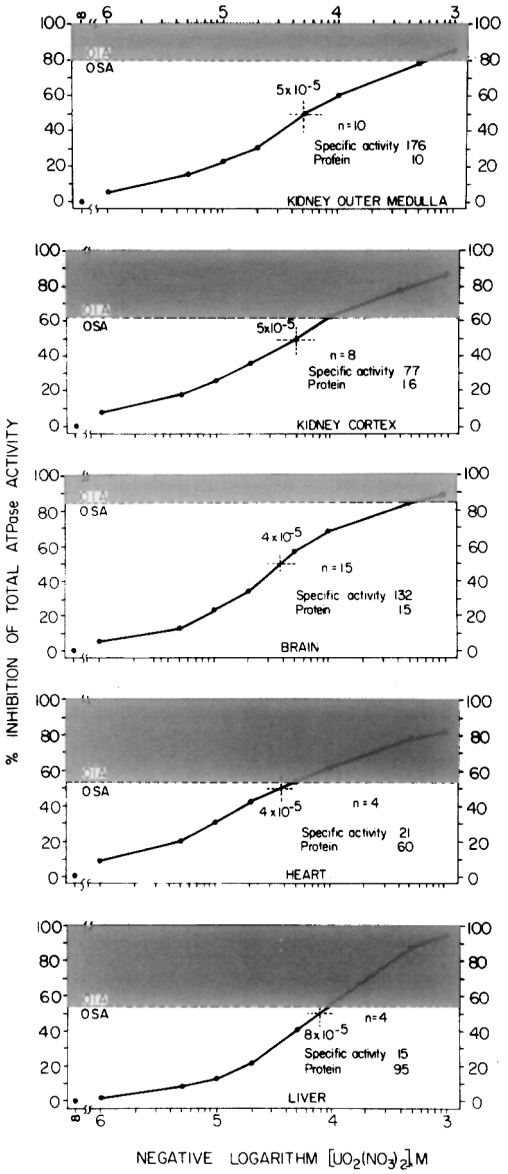

FIG. 1. Effects of U^{6+} on total ATPase activity in microsomal fractions of dog tissues. Shaded and clear areas in each graph separate ouabain-sensitive ($Na^+ + K^+$-dependent) ATPase activity (OSA) from ouabain-insensitive ATPase activity (OIA). The cross lines show molar concentrations of U^{6+} causing 50% inhibition of total ATPase activity. The symbol n indicates the number of experiments on tissues of at least four dogs. Average specific activity of total ATPase in the absence of U^{6+} is given in μmol P_i hr^{-1} mg $protein^{-1}$. Average protein content of the microsomal aliquot in each tube is given in μg.

digitalis drugs, which are specific inhibitors of this enzyme system (Nechay, 1977). For this reason, we examined in detail the interaction of uranyl nitrate with $Na^+ + K^+$ ATPase *in vitro*. More limited studies were performed with uranium trichloride (UCl_3) which decomposes in water to U^{4+} (Windholtz, 1976). In general uranium compounds are poor inhibitors of enzymes. Adenosine triphosphatase and alkaline phosphatase have been reported to be resistant to U^{6+} but acid phosphatase was moderately sensitive to the same form of uranium (Voegtlin and Hodge, 1949/1953). We are not aware of any previous studies on the effects of uranium on $Na^+ + K^+$ ATPase. Results of preliminary studies on this subject have been published (Nechay and Saunders, 1978).

METHODS

The enzyme was prepared from tissues obtained at the time of death after an overdose (60 mg/kg iv) of pentobarbital (dog and cat) or a blow to the head (hamster, rat, and rabbit). Brain tissue was a mixture of cortical white and gray matter; heart tissue was left ventricle. A human kidney in good condition became available for this study because it could not be transplanted. This kidney was removed from a trauma victim and was cold-perfused for 26 h in an organ preservation unit as described previously (Nechay *et al.*, 1975).

Methods for enzyme isolation were also described previously (Nechay and Nelson, 1970). Briefly, tissue homogenates were prepared 1:10 in sucrose solution containing tris(hydroxymethyl)aminomethane, disodium edetate (EDTA), and sodium deoxycholate. These homogenates were diluted with sucrose–tris solution for direct studies or they were used for obtaining microsomal fractions by differential centrifugation. The enzyme activity was measured by the rate of release of inorganic phosphate (P_i) from exogenous ATP at 37°C. The incubation mixture contained the enzyme (diluted tissue homogenates or microsomal fractions), 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 3 mM ATP, and histidine-imidazole buffer at pH 7.4. ATPase activity inhibited by 1×10^{-4} M ouabain (3×10^{-3} M for the rat enzyme) was considered to represent $Na^+ + K^+$ ATPase activity.

Cellular components in 78,000g fractions, similar to microsomal preparations used in this study, were

examined by electron microscopy in the previous laboratory of one (B.R.N.) of the authors. As shown in a typical micrograph there were many vesicular structures and longitudinal masses resembling brush borders or microvilli. No intact mitochondria or mitochondrial fragments could be identified (Palmer and Posey, 1970).

Uranyl nitrate ($UO_2(NO_3)_2$) was obtained from J. T. Baker Chemical Company, Phillipsburg, New Jersey and was certified to meet ACS standards for chemical reagents. Uranium trichloride (UCl_3) was purchased from K & K Laboratories, Plainview, New York. Aqueous solutions of these compounds were preincubated at 37°C with the enzyme plus electrolytes, but without ATP, for 30 min. This preincubation time was determined to give maximal enzyme inhibition for each concentration of uranium used in this study. This reaction was then started by addition of ATP.

RESULTS

Figure 1 shows that microsomal ATPase derived from different tissues of the dog was inhibited to the extent of 50% (I50) by similar U^{6+} concentrations ranging from 4 to 5×10^{-5} M for the kidney, brain, and heart to 8×10^{-5} M for the liver. Thus, the liver enzyme preparation was about two times more resistant to U^{6+} than the preparations from the other tissues ($p < 0.01$). This may be related to a protective effect of the high protein content in the aliquots of the liver preparation used in this study. However, this was not clearly seen in heart preparations which had an intermediate protein content. Variations in the ratio of ouabain-sensitive to ouabain-insensitive ATPases among enzyme preparations used did not appear to influence the I50 of U^{6+}.

On the basis of experiments in preparations with different fractions of ouabain-sensitive ATPase activity (shown in Fig. 1), the I50 of U^{6+} for $Na^+ + K^+$ ATPase is similar to that for total ATPase activity.

To test the possibility that some tissue components may influence the inhibition, the effects of U^{6+} on ATPase activity in tissue homogenates were determined. As shown in Fig. 2, the I50 values of U^{6+} were 8×10^{-5} to 1×10^{-4} M in the kidney and

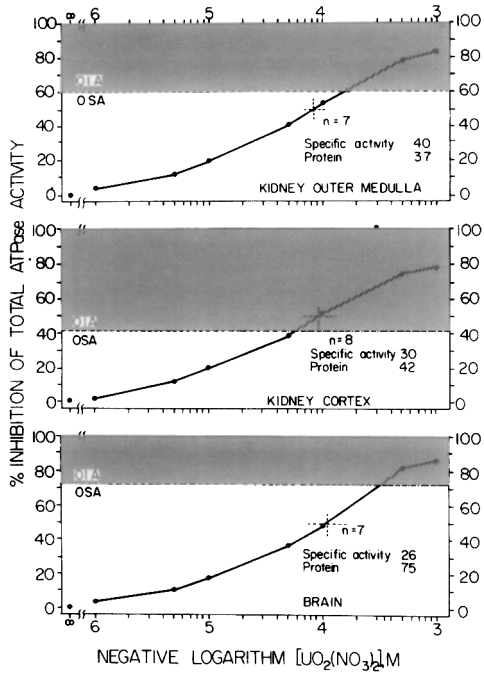

FIG. 2. Effects of U^{6+} on total ATPase activity in tissue homogenates of four dogs. Shaded and clear areas in each graph separate ouabain-sensitive ($Na^+ + K^+$-dependent) ATPase activity (OSA) from ouabain-insensitive ATPase activity (OIA). The cross lines approximate molar concentrations of U^{6+} causing 50% inhibition of total ATPase activity. Average specific activity of total ATPase is given in μmol P_i hr^{-1} mg $protein^{-1}$ in the absence of U^{6+}. Average protein content on the homogenate aliquot in each tube is given in μg.

brain homogenates. This is double ($p < 0.01$) the I50 values found in microsomes (4 to 5×10^{-5} M) derived from the same tissues (Fig. 1). These data suggest that homogenates of renal and brain tissue contain a substance(s) which interferes with the inhibition of ATPase by U^{6+}.

In the one human kidney available, the I50 values of U^{6+} in microsomes and homogenates of the whole organ were 1 and 2×10^{-4} M, respectively (Fig. 3). The corresponding renal values in the dog were 5×10^{-5} M (microsomes) and 8×10^{-5} to 1×10^{-4} M (homogenates). Thus, the human enzyme preparations were about

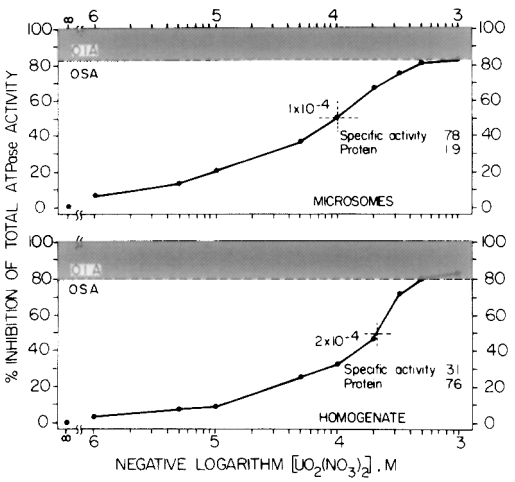

FIG. 3. Effects of U^{6+} on total ATPase activity in microsomes and homogenates of whole human kidney. Shaded and clear areas in each graph separate ouabain-sensitive ($Na^+ + K^+$-dependent) ATPase activity (OSA) from ouabain-insensitive ATPase activity (OIA). The cross lines approximate molar concentrations of U^{6+} causing 50% inhibition of total ATPase activity. Specific activity of total ATPase in the absence of U^{6+} is given in μmol P_i hr^{-1} $protein^{-1}$. Protein content of the microsomal or homogenate aliquot in each tube is given in μg.

twice as resistant to the action of U^{6+} as those of the dog.

The enzyme preparations used in this study evinced ouabain-sensitive ($Na^+ + K^+$) ATPase and ouabain-insensitive (Mg^{2+}) ATPase activities in various proportions. To compare the sensitivity of the two enzymes to U^{6+}, the effect of the metal was tested in dog (Fig. 4) and human (Fig. 5) enzyme preparations maximally inhibited by ouabain.

In the dog kidney and brain microsomes and kidney homogenates the I50 values of U^{6+} for Mg^{2+} ATPase were the same (Fig. 4) as those for total ATPase in corresponding preparations (Figs. 1 and 2). Because of lower dilutions there was more protein in each experiment with Mg^{2+} ATPase than with total ATPase. Therefore, the Mg^{2+} ATPase may be more sensitive to U^{6+} than the total ATPase if protein protects the enzyme from inhibition.

The upper part of Fig. 5 shows that in the human kidney microsomes, the I50 of U^{6+} for Mg^{2+} ATPase was 2×10^{-5} M or five times less than for total microsomal ATPase (1×10^{-4} M in Fig. 3). This obtains in spite of the fact that there was more protein present in experiments with Mg^{2+} ATPase than with total ATPase.

The bottom part of Fig. 5 shows that in human kidney homogenates the I50 of U^{6+} for Mg^{2+} ATPase was 5×10^{-4} M or 2.5 times more than for total ATPase in the same homogenates (2×10^{-4} M in Fig. 3). This difference can be attributed the presence of very high protein concentrations in experiments using Mg^{2+}-dependent enzyme.

Figure 6 shows that the inhibition of both the total and the Mg^{2+} ATPase activities by

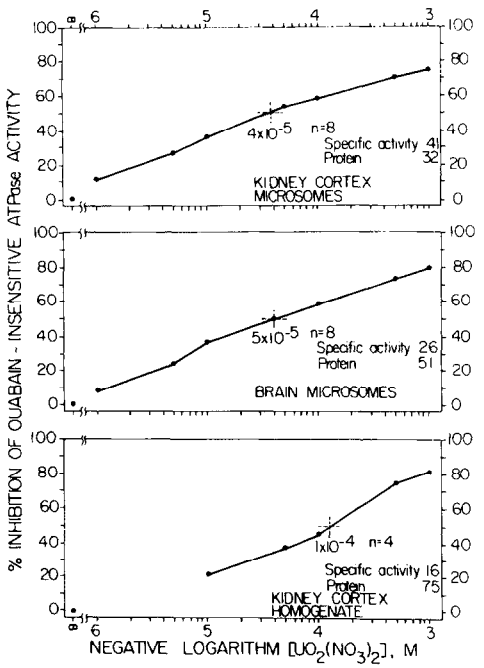

FIG. 4. Effects of U^{6+} on dog kidney and brain ATPase activity remaining after addition of 10^{-4} M ouabain. The cross lines approximate molar concentrations of U^{6+} causing 50% inhibition of ouabain-insensitive ATPase activity. The symbol *n* indicates the number of experiments on tissues of at least four dogs. Average specific activity of ouabain-insensitive ATPase in the absence of U^{6+} is given in μmol P_i hr^{-1} mg $protein^{-1}$. Average protein content of the enzyme aliquot in each tube is given in μg.

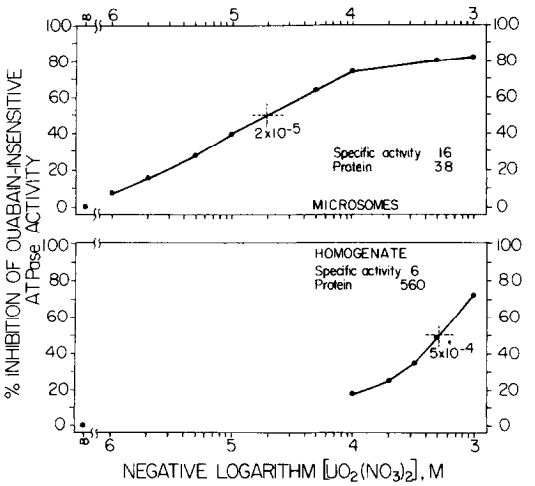

FIG. 5. Effects of U^{6+} on human kidney ATPase activity remaining after addition of 10^{-4} M ouabain. The cross lines approximate molar concentrations of U^{6+} causing 50% inhibition of ouabain-insensitive ATPase activity. Specific activity of ouabain-insensitive ATPase in absence of U^{6+} is given in μmol P_i hr^{-1} mg $protein^{-1}$. Protein content of the enzyme aliquot in each tube is given in μg.

U^{6+} is dependent on the concentration of protein in microsomal preparations. When protein influences are considered the Mg^{2+} ATPase is more sensitive to uranium than is the total ATPase. Since over 80% of total ATPase in this preparation is sensitive to ouabain (Fig. 4) these data indicate that $Na^+ + K^+$ ATPase is more resistant to uranium than is Mg^{2+} ATPase.

Dose–response curves were also determined in microsomal enzyme preparations from different organs of several other species. Table 1 presents a summary of these results and dog and human data from Figs. 1 to 5 are listed. In the cat, hamster, rabbit, and rat the results were similar to those in the dog and human. In human kidney microsomes, $Na^+ + K^+$ ATPase and Mg^{2+} ATPase exhibited the largest difference in sensitivity to uranium, the latter enzyme being more sensitive.

The remaining experiments were done to gain an insight into the mechanism of ATPase inhibition by uranium and to determine whether or not the inhibition could be modified by endogenous and exogenous agents.

To determine if the concentration of K^+ or Na^+ would influence the inhibition of $Na^+ + K^+$ ATPase activity by U^{6+}, the NaCl concentration was varied from 10 to 200 mM while the KCl concentration remained constant (20 mM); alternatively, KCl concentrations ranging from 2 to 20 mM were combined with a constant NaCl concentration (100 mM). Table 2 shows that in dog brain and human kidney preparations Na^+ antagonized the inhibition of $Na^+ + K^+$ ATPase by a constant concentration of U^{6+}. K^+ had no effect on the inhibition. This suggests that U^{6+} inhibits the enzyme in competition with Na^+ for the site activated by that cation or that U^{6+} alters allosterically the affinity of the enzyme for Na^+. To determine if this finding is specific for the $Na^+ + K^+$-activated enzyme analogous experiments were performed in preparations with all $Na^+ + K^+$ ATPase activity inhibited by ouabain. As shown in Table 3, Na^+ had only minimal protective effect and K^+ had no effect on inhibition of Mg^{2+} ATPase. Thus, the inhibition of Mg^{2+} ATPase appears to occur at a site different from that on $Na^+ + K^+$ ATPase.

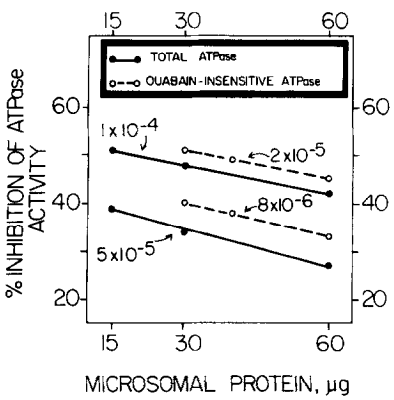

FIG. 6. Effects of fixed molar concentrations of uranyl nitrate (shown inside the graph) on ATPase inhibition at different amounts of microsomal protein. The source of the microsomes was human kidney.

TABLE 1

CONCENTRATIONS OF URANYL NITRATE REQUIRED TO INHIBIT 50% TOTAL ATPase AND Mg^{2+} ATPase ACTIVITIES IN VARIOUS ENZYME PREPARATIONS FROM DIFFERENT SPECIES

Species	No. of animals	Tissue	Sensitivity to ouabain (%)	Total ATPase activity: I50 $UO_2(NO_3)_2$ (M)	Total ATPase activity: Protein in assay (μg)	Mg^{2+} ATPase activity[a]: I50 $UO_2(NO_3)_2$ (M)	Mg^{2+} ATPase activity[a]: Protein in assay (μg)
Dog[b]		Kidney cortex (M)[c]	62	5×10^{-5}	16	4×10^{-5}	32
		Kidney outer medulla (M)	79	5×10^{-5}	10		
		Brain (M)	83	5×10^{-5}	15	5×10^{-5}	51
		Heart (M)	53	4×10^{-5}	60		
		Liver (M)	53	8×10^{-5}	95		
		Kidney cortex (H)	41	9×10^{-5}	42	1×10^{-4}	75
		Kidney outer medulla (H)	60	8×10^{-5}	37		
		Brain (H)	62	1×10^{-4}	75		
Man[b]		Whole kidney (M)	82	1×10^{-4}	19	2×10^{-5}	38
		Whole kidney (H)	80	2×10^{-4}	76	5×10^{-4}	560
Cat	2	Whole kidney (M)	53	1×10^{-4}	44		
		Brain (M)	81	5×10^{-5}	15	1×10^{-4}	30
		Heart (M)	39	2×10^{-4}	96		
Hamster	1	Whole kidney (M)	56	1×10^{-4}	21	2×10^{-4}	63
Rabbit	2	Whole kidney (M)	48	1×10^{-4}	19	2×10^{-4}	33
		Brain (M)	84	5×10^{-5}	20		
		Heart (M)	32	1×10^{-4}	65		
Rat	2	Whole kidney (M)	52	7×10^{-5}	9		
		Brain (M)	79	5×10^{-5}	12		

[a] Mg^{2+} ATPase refers to enzyme activity remaining after an addition of 10^{-4} M ouabain (3×10^{-3} *M* in case of preparations from the rat).

[b] Dog and human data are from Figs. 1 to 5.

[c] M, Microsomes; H, homogenate.

To test whether ATP or Mg^{2+} would influence the inhibition of $Na^+ + K^+$ ATPase activity by U^{6+}, experiments were conducted with concentration of ATP and $MgCl_2$ varying independently or together while the concentration of U^{6+} was kept constant. Table 2 shows that in dog brain and human kidney preparations, increasing ATP together with $MgCl_2$ (from 2 to 6 mM) reduced the inhibition. Increasing ATP alone (from 2 to 6 mM) while keeping the $MgCl_2$ concentration constant (3 mM), reduced the inhibition even more markedly. Keeping the ATP concentration constant (3 mM) while increasing $MgCl_2$ concentration from 3 to 9 mM enhanced the inhibition. Similar results were obtained in analogous experiments with Mg^{2+} ATPase preparations (Table 3). Thus, U^{6+} inhibits both ATPases probably at the ATP site.

To determine if albumin would modify the inhibition, bovine serum albumin was added together with U^{6+} to dog brain microsomes. Figure 7 shows that bovine serum albumin antagonized the inhibition of $Na^+ + K^+$ ATPase activity by U^{6+} in a concentration-dependent manner. Protection of the enzyme by albumin agrees with previous findings that U^{6+} complexes with -COOH groups of various proteins including albumin (Voegtlin and Hodge, 1949/1953).

Based on reports that citrate is one of the most powerful complexers of U^{6+} and reduces its toxicity (Voegtlin and Hodge,

TABLE 2

EFFECTS OF Na^+, K^+, ATP, AND Mg^{2+} ON INHIBITION OF ENZYME PREPARATIONS WITH PREDOMINANTLY $Na^+ + K^+$ ATPase ACTIVITY BY $UO_2(NO_3)$[a]

NaCl (mM)	KCl (mM)	ATP (mM)	$MgCl_2$ (mM)	Percentage inhibition of total ATPase: Dog brain microsomes 3×10^{-5} M $UO_2(NO_3)_2$	Percentage inhibition of total ATPase: Human kidney homogenate 2×10^{-4} M $UO_2(NO_3)_2$
10	20	3	3	49 ± 1	74 ± 3
20	20	3	3	44 ± 1	72 ± 2
30	20	3	3	40 ± 1	70 ± 2
50	20	3	3	38 ± 1	68 ± 2
100	20	3	3	33 ± 1	57 ± 1
200	20	3	3	24 ± 1	51 ± 1
100	2	3	3	35 ± 2	62 ± 3
100	4	3	3	36 ± 1	62 ± 1
100	8	3	3	36 ± 1	62 ± 2
100	12	3	3	36 ± 1	60 ± 1
100	16	3	3	34 ± 2	58 ± 2
100	20	3	3	32 ± 1	58 ± 3
100	20	2	2	77 ± 2	71 ± 2
100	20	4	4	60 ± 1	65 ± 2
100	20	6	6	55 ± 1	59 ± 2
100	20	1	3	66 ± 3	96 ± 1
100	20	2	3	48 ± 2	73 ± 1
100	20	3	3	36 ± 1	48 ± 1
100	20	6	3	24 ± 1	20 ± 1
100	20	3	3	38 ± 1	54 ± 1
100	20	3	6	59 ± 1	73 ± 1
100	20	3	9	67 ± 2	82 ± 1

[a] The results represent the mean ± SE of four experiments. The sensitivity to 10^{-4} M ouabain was 83% for the dog enzyme and 86% for the human enzyme. In each series of experiments the control enzyme activity remained within 20% of the entire range of NaCl, KCl, ATP, and $MgCl_2$ concentrations used.

1949/1953) we tested the effects of citrate on the inhibition of $Na^+ + K^+$ ATPase by uranium. Figure 8 shows that citrate protected the enzyme in a concentration-dependent manner. Equimolar concentrations of citrate with U^{6+} were not effective; 10- and 50-fold molar excess of citrate over U^{6+} reduced inhibition markedly.

Since bicarbonate reduces uranium toxicity (Voegtlin and Hodge, 1949/1953) we tested the effect of pH on inhibition of $Na^+ + K^+$ ATPase by uranyl nitrate. Over a pH range of 6.8 to 8.0 the inhibition by a constant concentration of U^{6+} increased with the alkalinity of the medium (Table 4).

On the expectation that ascorbic acid would reduce the oxidation state of uranium from 6+ (reacts with anions) to 4+ (reacts with anions and –SH), we tested the effect of ascorbic acid on the inhibition of $Na^+ + K^+$ ATPase by uranyl nitrate. Figure 9 shows that in the dog brain microsomes 10^{-4} and 10^{-5} M ascorbic acid increased the potency of uranyl nitrate in a concentration-dependent manner.

We attempted to determine the I50 for U^{4+} but that form of uranium produced inconsistent results. This is in agreement with difficulties encountered previously by others (Voegtlin and Hodge, 1949/1953).

TABLE 3

EFFECT OF Na^+, K^+, ATP, AND $MgCl_2$ ON INHIBITION OF Mg^{2+} ATPase BY $UO_2(NO_3)_2$[a]

NaCl (mM)	KCl (mM)	ATP (mM)	$MgCl_2$ (mM)	Percentage inhibition of Mg^{2+} ATPase: Dog brain microsomes 5×10^{-5} M $UO_2(NO_3)_2$	Percentage inhibition of Mg^{2+} ATPase: Human kidney homogenate 2×10^{-4} M $UO_2(NO_3)_2$
10	20	3	3	46 ± 2	64 ± 3
20	20	3	3	46 ± 2	64 ± 2
30	20	3	3	46 ± 2	62 ± 2
50	20	3	3	40 ± 3	60 ± 2
100	20	3	3	41 ± 3	59 ± 2
200	20	3	3	38 ± 4	58 ± 3
100	2	3	3	45 ± 3	55 ± 4
100	4	3	3	45 ± 3	55 ± 4
100	8	3	3	45 ± 3	51 ± 3
100	12	3	3	43 ± 3	52 ± 4
100	16	3	3	42 ± 3	55 ± 2
100	20	3	3	45 ± 3	52 ± 2
100	20	2	2	67 ± 1	51 ± 2
100	20	4	4	60 ± 1	44 ± 1
100	20	6	6	55 ± 1	38 ± 2
100	20	1	3	73 ± 3	66 ± 2
100	20	2	3	59 ± 1	58 ± 1
100	20	3	3	54 ± 2	50 ± 2
100	20	6	3	46 ± 2	34 ± 3
100	20	3	3	50 ± 2	43 ± 1
100	20	3	6	62 ± 1	51 ± 1
100	20	3	9	68 ± 2	64 ± 1

[a] The results represent the mean ± SE of five experiments. All experiments were performed with $Na^+ + K^+$ ATPase inhibited by 10^{-4} M ouabain. In each series of experiments the control enzyme activity remained within 20% of the entire range of NaCl, KCl, ATP, and $MgCl_2$ concentrations used.

Specifically, we used UCl_3 which decomposes in water solutions to U^{4+} (Windholtz, 1976). In dog brain microsomes the I50 values ranged widely from 5×10^{-6} to 3×10^{-5} M.

DISCUSSION

Under our test conditions U^{6+} is an inhibitor of both $Na^+ + K^+$ ATPase and Mg^{2+} ATPase activities at concentrations on the order of 10^{-5} to 10^{-4} M. The Mg^{2+}-dependent enzyme appears to be somewhat more sensitive to U^{6+} than the $Na^+ + K^+$ ATPase.

U^{6+} inhibits $Na^+ + K^+$ ATPase by a mechanism different from that exerted by cardiac glycosides. In the latter case K^+ competes with ouabain for a site on the enzyme (Skou, 1965) while Na^+ protects the enzyme from inhibition by U^{6+}; thus, U^{6+} either binds to the Na^+ site on the enzyme or acts allosterically. K^+ is without any effect on the inhibition of $Na^+ + K^+$ ATPase by U^{6+}.

U^{6+} inhibition of $Na^+ + K^+$ ATPase and Mg^{2+} ATPase also appears to be different since the interaction of U^{6+} with the latter enzyme is scarcely modified by Na^+.

The present data do not establish the mechanism for the protection of $Na^+ + K^+$ ATPase and Mg^{2+} ATPase by ATP. The obvious possibilities are a competition of U^{6+}

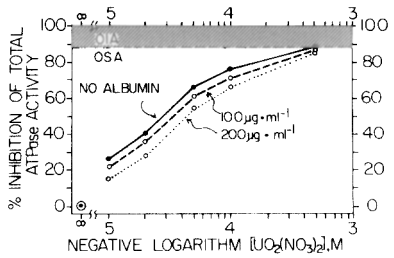

FIG. 7. Effect of bovine serum albumin on total ATPase inhibition by U^{6+}. The source of the enzyme was the microsomal fraction from dog brain. Shaded and clear areas in the graph separate ouabain-sensitive ($Na^+ + K^+$-dependent) ATPase activity (OSA) from ouabain-insensitive ATPase activity (OIA). Bovine serum albumin was preincubated with uranyl nitrate in a mixture of buffer and electrolytes at 37°C for 30 min before ATP was added to start the enzyme reaction. Each curve represents three experiments on three different enzyme preparations.

with ATP for the receptor site on the enzyme or the binding of U^{6+} to ATP. U^{6+} is known to bind ATP (Voegtlin and Hodge, 1949/1953). A 10- to 100-fold excess of ATP over U^{6+} is required to observe enzyme protection under our test conditions.

These observations on the inhibition of $Na^+ + K^+$ ATPase *in vitro* are pertinent to the understanding of impaired Na^+Cl^- transport which results in the diuresis and

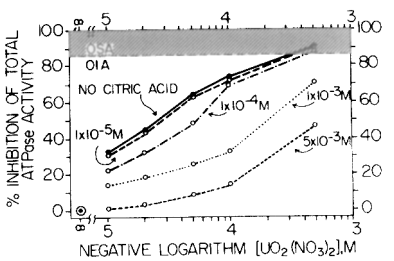

FIG. 8. Effect of citric acid on total ATPase inhibition by U^{6+}. The source of the enzyme was the microsomal fraction from dog brain. Shaded and clear areas in the graph separate ouabain-sensitive ($Na^+ + K^+$-dependent) ATPase activity (OSA) from ouabain-insensitive ATPase activity (OIA). Citric acid was preincubated with uranyl nitrate in a mixture of buffer and electrolytes at 37°C for 30 min before ATP was added to start the enzyme reaction. Each curve represents four experiments on four different enzyme preparations.

TABLE 4

EFFECT OF pH ON INHIBITION OF AN ENZYME PREPARATION WITH PREDOMINANTLY $Na^+ + K^+$ ATPase ACTIVITY BY 3×10^{-5} M $UO_2(NO_3)_2$[a]

pH	Percentage inhibition of total ATPase
6.8	39 ± 3
7.4	44 ± 2
8.0	58 ± 2

[a] The results represent the mean of five experiments ± SE. The sensitivity to 10^{-4} M ouabain of these dog brain microsomal preparations was 85%. The control enzyme activity remained within 10% at pH 7.4 and 8.0; it was 25% lower at pH 6.8.

defects in urinary dilution and concentration observed in U^{6+} poisoning (Bowman and Foulkes, 1970). However, they can not be quantitatively extrapolated to the situation *in vivo* because reactants that govern $Na^+ + K^+$ ATPase activity, such as Na^+, Mg^{2+}, and ATP modify the inhibition of the enzyme by U^{6+}. The inhibition may also be altered by albumin, citrate, H^+, and unidentified tissue components. In addition, numerous other endogenous anions such as bicarbonate, phosphates, and carboxylic acids which are known to complex with U^{6+} (Hodge *et al.*, 1973; Voegtlin and Hodge, 1949/1953) would be expected to modify transport, distribution, and behavior of the

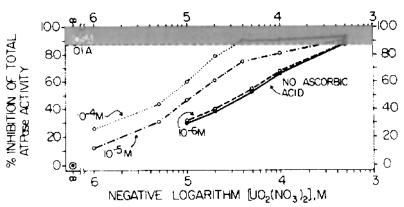

FIG. 9. Effect of ascorbic acid on total ATPase inhibition by U^{6+}. The source of the enzyme was the microsomal fraction from dog brain. Shaded and clear areas in the graph separate ouabain-sensitive ($Na^+ + K^+$-dependent) ATPase activity (OSA) from ouabain-insensitive ATPase activity (OIA). Ascorbic acid was preincubated with uranyl nitrate in a mixture of buffer and electrolytes at 37°C for 30 min before ATP was added to start the enzyme reaction. Each curve represents three experiments on three different enzyme preparations.

metal *in vivo*. An interesting situation arises with respect to acid-base balance. While the administration of sodium bicarbonate may serve as an antidote for U^{6+} poisoning by increasing urinary excretion of U^{6+} in experimental animals (Hodge *et al.*, 1973; Voegtlin and Hodge, 1949/1953), decreasing H^+ enhances the *in vitro* inhibition of $Na^+ + K^+$ ATPase by U^{6+}.

The significance of inhibition of microsomal Mg^{2+} ATPase remains unclear, for the function of this enzyme in the kidney has not yet been elucidated.

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