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METABOLIC EFFECTS OF ZINC IN INTACT CELLS—COMPARATIVE STUDIES OF ZINC CHLORIDE AND THE ZINC CHELATE OF KETHOXAL BIS(THIOSEMICARBAZONE)*

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Abstract—Kethoxal bis(thiosemicarbazone)Zinc(II) [Zn(II)KTS] and zinc chloride were both cytotoxic to sarcoma 180 ascites cells; equivalent quantities of zinc administered in chelate form were considerably more toxic to the neoplastic cells. This correlated with the finding of greater accumulation of zinc by cells treated with Zn(II)KTS, as compared to those exposed to $ZnCl_2$. In general, however, the metabolic consequences of $ZnCl_2$ appeared to be greater than those of Zn(II)KTS. The biosynthesis of DNA as measured by the incorporation of [^{3}H]thymidine and [8-^{14}C]adenine into DNA was markedly sensitive to both agents; although thymidine was converted to the triphosphate level at a rate comparable to controls in cells in which the biosynthesis of DNA was initially inhibited by Zn(II)KTS or $ZnCl_2$, at later times after drug treatment the degree of phosphorylation of thymidine was markedly depressed. Shortly after exposure of cells to Zn(II)KTS, the incorporation of [6-^{14}C]orotic acid into RNA was strongly inhibited, while the utilization of [5,6-^{3}H]uridine and [U-^{14}C]aspartic acid for the biosynthesis of these macromolecules was unaffected. In contrast, $ZnCl_2$ initially caused inhibition of the incorporation of [^{3}H]uridine into RNA, concomitant with pronounced stimulation of the conversion of [6-^{14}C]orotic acid and [^{14}C]aspartic acid to RNA. Inhibition of the incorporation of all labeled precursors into RNA accompanied prolonged exposure to either of these agents. Significant blockage of equal magnitude of the incorporation of [1-^{14}C]leucine into protein and of glycolysis was produced by Zn(II)KTS or $ZnCl_2$; however, Zn(II)KTS caused greater inhibition than $ZnCl_2$ of the respiration of sarcoma 180 cells.

THE CARCINOSTATIC POTENCY of 2-keto-3-ethoxybutyraldehyde bis(thiosemicarbazone) [kethoxal bis(thiosemicarbazone; KTS] against transplanted rodent tumors has been well documented.[1–9] In the presence of either copper or zinc ions the therapeutic potency of KTS, an agent that forms tetradentate chelates with metals of the first, second and third transition series, is markedly elevated.[8–13]

Studies on the biochemical mechanism of action of KTS and its cupric chelate [Cu(II)KTS] have been described.[11,14–18] These agents primarily inhibit the synthesis of DNA; the rate of formation of RNA and protein is also depressed, but these latter processes are less affected. KTS, at concentrations that are achieved intracellularly by tumor cells, is inhibitory to thymidylate synthetase, 5,10-methylene tetrahydrofolate dehydrogenase and dihydrofolate reductase;[17] such blockade apparently accounts for the inhibition of the biosynthesis of DNA by this agent.

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The cellular toxicity of Cu(II)KTS, however, appears to be due primarily to copper. Cu(II)KTS, a relatively lipid-soluble form of copper, serves effectively to transport this metal to sensitive cellular sites. Apparently greater affinity of intracellular components for copper leads to the dissociation of the chelate and deposition of the metal within tumor cells. The ligand portion of the molecule, KTS, is rapidly eliminated from the cell, while the copper is retained for a much longer period. The use of several radioactive precursors of DNA led to findings suggesting that the intracellular localization of copper from Cu(II)KTS differed to some extent from that of $CuCl_2$.[16]

Since little is known of the molecular mechanism of action of the zinc chelate of KTS [Zn(II)KTS], the present report compares the toxic effects of this agent with $ZnCl_2$ on several biochemical processes in sarcoma 180 ascites cells.

MATERIALS AND METHODS

Experiments were performed on 9–11-week-old female CD-1 mice (Charles River Breeding Laboratories, North Wilmington, Mass.) bearing sarcoma 180 ascites cells. Transplantation of the tumor was carried out by withdrawing peritoneal fluid from a donor mouse bearing a 7-day growth. The suspension was centrifuged for 2 min (1600 *g*), the supernatant peritoneal fluid was decanted, a 15-fold dilution with isotonic saline was made, and 0·1 ml of the resulting cell suspension was injected intraperitoneally into each animal.[19]

Zinc chloride was dissolved in isotonic saline; Zn(II)KTS and KTS were suspended by homogenization in absolute ethanol (final concentration of the drug suspension was 5 per cent with respect to ethanol) and 2–3 drops of 20 per cent aqueous Tween 80, and then made up to volume with isotonic saline. A volume of 0·5 ml was administered by i.p. injection to drug-treated mice.

Biochemical studies were conducted with animals bearing 6-day growths of sarcoma 180. A single dose of drug was given at selected periods of time before the mouse received an i.p. injection of either 200 μg of [^{3}H]methyl thymidine ($5{\cdot}7 \times 10^3$ counts/min/μg), 100 μg of [6-^{14}C]orotic acid hydrate ($2{\cdot}1 \times 10^4$ counts/min/μg), 125 μg of [1-^{14}C]DL-leucine ($1{\cdot}4 \times 10^4$ counts/min/μg), 90 μg of [^{14}C]sodium formate ($2{\cdot}9 \times 10^4$ counts/min/μg), 200 μg of [5,6-^{3}H]uridine ($7{\cdot}1 \times 10^3$ counts/min/μg), 50 μg of [8-^{14}C]adenine ($2{\cdot}9 \times 10^4$ counts/min/μg), 660 μg of [^{3}H]deoxycytidine ($1{\cdot}1 \times 10^3$ counts/min/μg), or 150 μg of [U-^{14}C]L-aspartic acid ($5{\cdot}8 \times 10^4$ counts/min/μg); 1 hr was allowed for metabolism of radioactive precursors.

In experiments involving the incorporation of either [^{3}H]thymidine, [6-^{14}C]orotic acid, [^{14}C]formate, or [^{3}H]deoxycytidine into nucleic acids, sodium nucleates were isolated by the method of Tyner *et al.*[20] and hydrolyzed for 1·5 hr with 70% perchloric acid (PCA).[21] Extracts were purified and analyzed as described by Danneberg *et al.*[22] With [^{14}C]adenine and [^{3}H]uridine, the dried sodium nucleates were subjected to alkaline hydrolysis, the DNA was precipitated by acidification and separated from RNA by centrifugation. When [^{14}C]adenine was employed, the purine nucleotides of both fractions were hydrolyzed with acid, and the bases were purified and analyzed.[23] With [^{3}H]uridine, aliquots of PCA-hydrolyzed DNA and RNA were analyzed both for radioactivity and for deoxyribose and ribose, using deoxyadenosine and adenosine, respectively, as the standards.[24]

Residual protein was isolated after exposure of cells to [^{14}C]leucine and analyzed as

previously reported.[25] Radioactivity in protein was measured with a Nuclear-Chicago gas flow counter, and in all other instances with a Packard Tri-Carb liquid scintillation spectrometer. The phosphor solution contained 100 mg of *p*-bis[2-(5-phenyloxazolyl)]benzene and 8 g of 2,5-diphenyloxazole dissolved in a mixture of 2 liters of toluene and 1 liter of absolute ethanol.

Respiration and anaerobic glycolysis were measured in sarcoma 180 cells freed of erythrocytes by brief exposure to 0·2% NaCl using a Gilson Differential Respirometer. Two hundred milligrams of cells wet weight in a volume of 3 ml of cold phosphate saline[26] were added to 10-ml Warburg flasks containing 0·2 ml of 1 N KOH in the center well. Cells were equilibrated in 95% O_2 : 5% CO_2 for 5 min at 37° before measurements were taken; oxygen uptake was followed at 5-min intervals for 30 min. For determination of the glycolytic rate, 200 mg of cells wet weight were incubated in 2·7 ml of Krebs–Ringer bicarbonate in 10-ml Warburg flasks containing 0·3 ml of 0·055 M glucose in a side arm. Flasks were equilibrated in 95% N_2 : 5% CO_2 for 5 min at 37°, glucose present in the side arm was added, and CO_2 production was measured at 5-min intervals for 30 min.

The cellular concentration of zinc was measured using a Perkin–Elmer Atomic Absorption Spectrophotometer following wet ashing of the tissue. Total cells in the peritoneal cavity were measured by quantitatively washing the cells out of the cavity and determining the cell number using a Coulter particle counter, model A. The average cell volumes were measured in hematocrit tubes by determining the volume occupied by a known number of cells.

Cell-free extracts were prepared from 6-day growths of ascites cells that were exposed to either $ZnCl_2$ or Zn(II)KTS for various periods of time, and the ability of these extracts to convert [^{3}H]thymidine to thymine nucleotides was measured as previously described.[27]

RESULTS

The comparative toxicity of molar equivalent quantities of $ZnCl_2$ (20 mg/kg) and Zn(II)KTS (50 mg/kg) to sarcoma 180 ascites cells was determined by measuring the total number of neoplastic cells present in the peritoneal cavities of mice 12 and 24 hr after a single dose of drug. The data are shown in Table 1. Twenty-four hours

TABLE 1. TOXICITY OF Zn(II)KTS AND $ZnCl_2$ TO SARCOMA 180 ASCITES CELLS*

Treatment	Concentration (mg/kg)	Time after drug (hr)	Total cells/ mouse $\times 10^{-6}$	Cell volume μ^3/cell $\times 10^{-3}$
None		12	529 ± 24	3·28 ± 0·3
		24	653 ± 67	3·09 ± 0·6
$ZnCl_2$	20	12	510 ± 53	5·01 ± 0·7
		24	404 ± 66	6·44 ± 0·8
Zn(II)KTS	50	12	449 ± 52	5·79 ± 1·1
		24	189 ± 46	12·93 ± 2·4

* Twelve and 24 hr before collection of cells, mice were injected i.p. with either $ZnCl_2$ or Zn(II)KTS. Ascites cells were quantitatively collected from the peritoneal cavities, and the numbers and average mean volume of the population were determined. Each value represents the mean ± S.E. of results from the separate analyses of 8 animals.

after exposure to Zn(II)KTS a 71 per cent decrease was obtained in the number of cells present in the peritoneal cavity, whereas $ZnCl_2$ caused only a 38 per cent loss in cell number. An increase in the average volume of the cell population accompanied the loss in cell number, with the greater increase occurring in cells exposed to Zn(II)KTS.

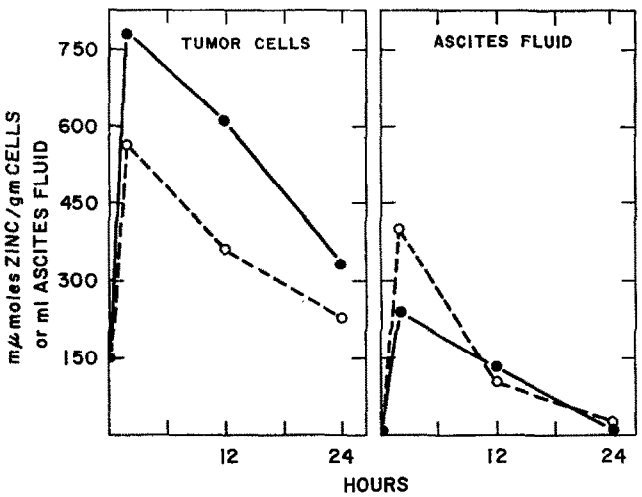

FIG. 1. Zinc content of sarcoma 180 cells and ascites fluid after exposure to either Zn(II)KTS or $ZnCl_2$. At various times after administration of either Zn(II)KTS (50 mg/kg) or $ZnCl_2$ (20 mg/kg), ascites fluid was collected. The zinc content of the cells and of the fluid was determined. Each value represents the mean obtained from the separate analyses of 2–4 animals. Zn(II)KTS, ●——● ; $ZnCl_2$, ○— — —○.

The amount of zinc assimilated and retained by neoplastic cells following exposure of tumor-bearing animals to either Zn(II)KTS or $ZnCl_2$ was determined (Fig. 1). Two hours after a single dose of Zn(II)KTS, tumor cells contained about 30 per cent more zinc than those receiving an equivalent number of gram-atoms of $ZnCl_2$ under identical conditions. This was paralleled by a lower concentration of zinc in the ascites fluid of animals 2 hr after treatment with Zn(II)KTS as compared to the fluid from those mice receiving $ZnCl_2$. The rate of loss of zinc from sarcoma 180 cells was similar for both $ZnCl_2$- and Zn(II)KTS-treated cells.

The incorporation of [^{3}H]thymidine and [8-^{14}C]adenine into the thymine and adenine, respectively, of DNA was used to measure the effects of the inhibitors on the formation of these macromolecules (Fig. 2). The biosynthesis of DNA was extremely sensitive to both $ZnCl_2$ and Zn(II)KTS, with the duration of blockade by $ZnCl_2$ being more prolonged. The incorporation of thymidine into DNA was inhibited to a greater degree by both agents than was the utilization of adenine for the synthesis of these macromolecules.

The effect of Zn(II)KTS and $ZnCl_2$ on the ability of sarcoma 180 ascites cells to convert [^{3}H]thymidine to nucleotide forms is shown in Table 2. At 2 hr after exposure to $ZnCl_2$, and at both 2 and 12 hr after Zn(II)KTS, the neoplastic cells converted thymidine to its nucleotide forms in a manner comparable to untreated cells; however,

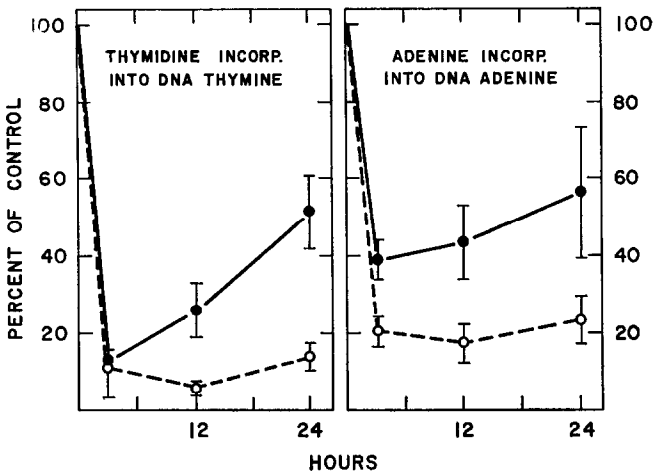

FIG. 2. Comparative effects of Zn(II)KTS and $ZnCl_2$ on the incorporation of [^{3}H]thymidine and [^{14}C]adenine into the thymine and adenine of DNA, respectively. At various times before the i.p. administration of [^{3}H]thymidine or [8-^{14}C]adenine, either Zn(II)KTS (50 mg/kg) or $ZnCl_2$ (20 mg/kg) was given by i.p. injection to each mouse. Each value represents the mean obtained from the separate analyses of ascites cells from 3–24 animals. The specific activity ± S.E. of non-drug-treated controls was 47·2 ± 3·7 counts/min/μmole × 10^{-2} and 36·0 ± 2·0 counts/min/μmole × 10^{-2} for [^{3}H]thymidine and [^{14}C]adenine, respectively. Zn(II)KTS, ●——●; $ZnCl_2$, ○— — —○.

by 24 hr after these agents, pronounced inhibition of incorporation of thymidine into nucleotides occurred.

The results presented in Table 3 show that Zn(II)KTS and $ZnCl_2$ at concentrations of 10^{-5} M caused little or no direct inhibition of the activities of thymidine kinase and thymidine monophosphate kinase; in contrast, thymidine monophosphate phosphohydrolase activity was completely inhibited by these agents.

The effects of Zn(II)KTS and $ZnCl_2$ on the syntheses of RNA and protein were

TABLE 2. EFFECT OF Zn(II)KTS AND $ZnCl_2$ ON THE METABOLISM OF [^{3}H]THYMIDINE TO NUCLEOTIDES IN SARCOMA 180 ASCITES CELLS*

Inhibitor	Time (hr)	Counts/min /g cells × 10^{-3}	Per cent of total acid-soluble radioactivity				Total per cent phosphorylated
			TdR	dTMP	dTDP	dTTP	
None		91·80	64·0	5·1	18·0	12·8	35·9
Zn(II)KTS	2	161·02	55·9	6·6	8·9	28·6	44·1
	12	99·08	65·5	4·3	9·5	20·8	34·6
	24	71·62	82·6	3·3	9·7	4·4	17·4
$ZnCl_2$	2	140·04	63·2	4·0	13·8	19·0	36·8
	12	85·78	76·3	7·1	6·3	10·4	23·8
	24	98·98	80·2	2·0	8·3	9·6	19·9

* Mice bearing 6-day growths of sarcoma 180 ascites cells were injected i.p. with either Zn(II)KTS (50 mg/kg) or $ZnCl_2$ (20 mg/kg). At the indicated times, [^{3}H]thymidine (200 μg/animal; 5·7 × 10^3 counts/min/μg) was administered and 1 hr was allowed for metabolism. Each value represents the mean results from 4 mice.

TABLE 3. EFFECT OF Zn(II)KTS and $ZnCl_2$ ON THE ACTIVITIES OF THYMIDINE KINASE, THYMIDINE MONOPHOSPHATE KINASE AND THYMIDINE MONOPHOSPHATE PHOSPHOHYDROLASE*

Inhibitor	Activity, mμmoles/mg/hr		
	Thymidine kinase	Thymidine monophosphate kinase	Thymidine monophosphate phosphohydrolase
None	28·3	40·2	5·3
Zn(II)KTS	24·7	49·7	0·0
$ZnCl_2$	20·4	44·0	0·0

* Zn(II)KTS and $ZnCl_2$ were employed at a concentration of 10^{-5} M. Zn(II)KTS was dissolved with the aid of ethanol; the final concentration of ethanol was 1·1%. This quantity of alcohol did not inhibit the activity of these enzymes. Each value represents the mean of duplicate flasks.

measured to determine whether drug-induced lesions occurred in other metabolic areas. The results presented in Fig. 3 show the action of Zn(II)KTS and $ZnCl_2$ on the incorporation of [^{3}H]uridine and [6-^{14}C]orotic acid into RNA. A marked depression of the utilization of uridine for the formation of RNA occurred in cells treated with $ZnCl_2$, whereas delayed and lesser blockade of uridine uptake into RNA was observed in neoplastic cells exposed to Zn(II)KTS. A different pattern was produced when orotic acid was employed as the measure of RNA biosynthesis. Approximately 90 per cent inhibition of the conversion of orotic acid to the uracil moiety of RNA was evident at 2 hr after Zn(II)KTS; partial recovery to 50 per cent of the control level occurred by 24 hr after the metal chelate. In contrast, a 2-hr exposure to $ZnCl_2$ caused

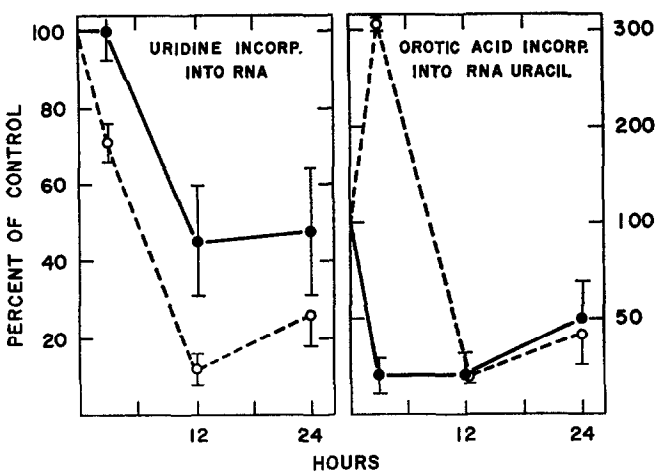

FIG. 3. Comparative effects of Zn(II)KTS and $ZnCl_2$ on the incorporation of [^{3}H]uridine and [^{14}C]orotic acid into RNA. At various times before the i.p. administration of [^{3}H]uridine or [6-^{14}C]-orotic acid, either Zn(II)KTS (50 mg/kg) or $ZnCl_2$ (20 mg/kg) was given by i.p. injection to each mouse. Each value represents the mean obtained from the separate analyses of ascites cells from 4–31 animals. The specific activity ± S.E. of non-drug-treated controls was 4·7 ± 0·3 counts/min/mμmole and 26·3 ± 1·9 counts/min/μmole × 10^{-2} for [^{3}H]uridine and [^{14}C]orotic acid, respectively. Zn(II)KTS, ●——●; $ZnCl_2$, ○— — —○.

a marked increase (> 300% of control) in the specific activity of RNA uracil. By 12 hr after the $ZnCl_2$, however, pronounced inhibition, comparable to that observed with Zn(II)KTS, was obtained. The action of these compounds on the incorporation of [^{14}C]aspartic acid into RNA was determined at 2 hr after treatment of sarcoma 180 tumor-bearing animals with either Zn(II)KTS (50 mg/kg) or $ZnCl_2$ (20 mg/kg). No decrease in the rate of utilization of aspartate for the formation of RNA was produced by these agents; a stimulation of 162 per cent of the control value was produced by $ZnCl_2$, while Zn(II)KTS did not significantly alter the rate of incorporation of aspartate into RNA.

Measurement of the uptake of [6-^{14}C]orotic acid by cells treated 2 hr previously with either Zn(II)KTS (50 mg/kg) or $ZnCl_2$ (20 mg/kg) showed that Zn(II)KTS caused an increase (172 per cent of control uptake) in the degree of accumulation of orotic acid by sarcoma 180 cells. An even greater increase to 226 per cent of the control value was produced in cells exposed to the inorganic form of zinc ($ZnCl_2$).

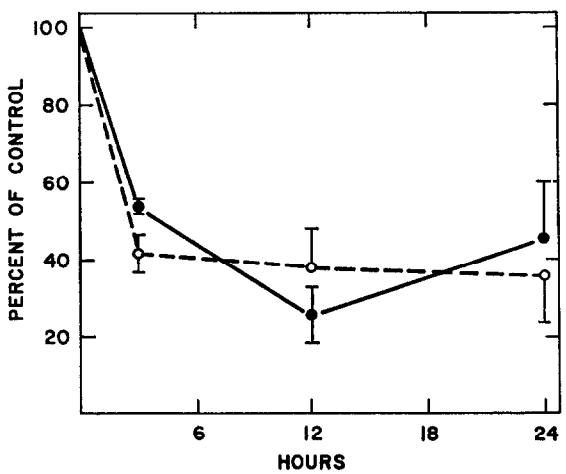

FIG. 4. Comparative effects of Zn(II)KTS and $ZnCl_2$ on the incorporation of [1-^{14}C]leucine into protein. At various times before the i.p. administration of [1-^{14}C]leucine, either Zn(II)KTS (50 mg/kg) or $ZnCl_2$ (20 mg/kg) was given by i.p. injection to each mouse. Each value represents the mean obtained from the separate analyses of ascites cells from 4–15 animals. A specific activity ± S.E. of 34·0 ± 3·5 counts/min/mg × 10^{-2} was obtained for untreated control cells. Zn(II)KTS, ●——●; $ZnCl_2$, ○— — —○.

No differences in the degree of inhibition of protein synthesis as measured by the fixation of [^{14}C]leucine into protein (Fig. 4) nor of glycolysis (Table 4) was produced by molar equivalent levels of Zn(II)KTS and $ZnCl_2$; however, greater interference with respiratory processes of sarcoma 180 cells was caused by Zn(II)KTS (Table 5), although the degree of inhibition of respiration was slight as compared to the effects of the metal chelate on the biosynthesis of nucleic acids and proteins.

DISCUSSION

Zinc ions have been reported to produce both stimulation[28–31] and inhibition[29,30,32] of nucleic acid synthesis in mammalian systems. Enhancement of the rate of formation

TABLE 4. EFFECT OF Zn(II)KTS AND $ZnCl_2$ TREATMENT ON THE GLYCOLYTIC RATE OF SARCOMA 180 ASCITES CELLS*

Time after inhibitor (hr)	Per cent control rate	
	Zn(II)KTS	$ZnCl_2$
6	57·3 ± 3·5	51·8 ± 5·3
12	50·3 ± 5·2	50·6 ± 5·4
18	64·0 ± 13·3	51·0 ± 11·0

* At various times after i.p. treatment of 6-day tumor-bearing animals with either Zn(II)KTS (50 mg/kg) or $ZnCl_2$ (20 mg/kg), cells were collected. Anaerobic glycolysis was measured in 95% N_2:5% CO_2 in a Warburg respirometer. Each value represents the mean ± S.E. of results from the separate analyses of cells from 3–7 animals. The $Q_{CO_2}^{N_2}$ of untreated sarcoma 180 cells was 16·1.

of DNA by added zinc ions required the initial production of a deficiency of this metal,[28,29] suggesting that zinc has a role in the biosynthesis of these macromolecules. Relatively high levels of zinc ions, however, cause inhibition of the synthesis of DNA, with DNA polymerase being the site of a sensitive enzymic reaction.[32] The results presented in this report with sarcoma 180 are in accord with the observation that the incorporation of radioactive precursors into DNA is sensitive to the toxic action of zinc, since exposure of cells to this metal in the form of either a salt or a coordination complex produced inhibition of the formation of DNA. The drug-induced decrease in the incorporation of thymidine into DNA by both Zn(II)KTS and $ZnCl_2$ at 2 hr after exposure of cells to these agents was not the result of interference with the conversion of thymidine to the triphosphate level (Table 2); however, pronounced inhibition of thymidine triphosphate synthesis from thymidine occurred at relatively long times after treatment of cells with zinc. That such inhibition was not the result of a direct effect on either thymidine kinase or thymidine monophosphate kinase was shown by the lack of sensitivity of these enzymes to added Zn(II)KTS or $ZnCl_2$ (Table 3); the lack of sensitivity of these enzymes to kethoxal bis(thiosemicarbazone)

TABLE 5. EFFECT OF Zn(II)KTS AND $ZnCl_2$ TREATMENT ON RESPIRATION OF SARCOMA 180 ASCITES CELLS*

Time after inhibitor (hr)	Per cent control rate		
	Zn(II)KTS	$ZnCl_2$	KTS
3	76·4 ± 5·2	79·4 ± 3·5	76·8 ± 2·3
6	69·5 ± 4·7	90·0 ± 4·4	86·8 ± 3·5
12	72·2 ± 3·6	92·5 ± 3·8	96·0†
18	100	90·5 ± 3·5	—‡

* At various times after i.p. treatment of 6-day tumor-bearing animals with either Zn(II)KTS (50 mg/kg), $ZnCl_2$ (20 mg/kg), or KTS (200 mg/kg), cells were collected. Respiration was measured in 95% O_2:5% CO_2 in a Warburg respirometer. Each value represents the mean ± S.E. of results from the separate analyses of cells from 4–9 animals. The Q_{O_2} of untreated sarcoma 180 cells was −7·1.

† This value represents the results from 2 animals only.

‡ Not determined.

(KTS), the ligand portion of the metal chelate, has been shown earlier.[16] The pronounced depression *in vivo* of the conversion of thymidine to phosphorylated derivatives by Zn(II)KTS at 24 hr after the drug (Table 2) is not in accord with the finding that the incorporation of thymidine into the thymine of DNA is inhibited only 50 per cent under these conditions (Fig. 1). One possible explanation for this phenomenon is that dissociation of the metal chelate occurs *in vivo*, as shown for Cu(II)KTS,[16] and inhibition of the KTS-sensitive enzymes thymidylate synthetase, 5,10-methylenetetrahydrofolate dehydrogenase and/or dihydrofolate reductase occurs.[11] Such lesions would be expected to depress the intracellular pool of thymidine triphosphate, the direct precursor of DNA; even though the rate of phosphorylation of thymidine is depressed, the resultant specific activity of the thymine nucleotide pool might well be increased to produce greater labeling of DNA.

In cells treated 2 hr previously with Zn(II)KTS no inhibition of the incorporation of uridine and aspartate into RNA occurred, while the conversion of orotic acid to uracil of RNA was markedly depressed. These findings suggest the presence of a sensitive enzyme involved in the metabolism of orotic acid to the nucleotide level; blockade can be envisioned to occur either directly at the level of the phosphoribosyl transferase enzyme, or at a reaction involved in the generation of phosphoribosylpyrophosphate. At 2 hr after treatment of cells with $ZnCl_2$, the incorporation of uridine into RNA was slightly depressed, whereas the specific radioactivity of RNA from orotic acid, and to a lesser extent from aspartate, was markedly increased (Fig. 3). Such apparent stimulation of the synthesis of RNA by $ZnCl_2$ may represent increased uptake of orotic acid in the form of a zinc complex, since the accumulation of radioactivity from orotate in sarcoma 180 cells was greatly enhanced by the presence of zinc ions. Stimulation of the synthesis of RNA in mammalian systems by zinc has, however, been reported.[29,31]

Both Zn(II)KTS and $ZnCl_2$ caused pronounced inhibition of the formation of RNA, measured with either [^{3}H]uridine or [^{14}C]orotic acid, at 12 and 24 hr after exposure of neoplastic cells to these agents. A known site of sensitivity to zinc ions in the RNA biosynthetic pathways is the DNA-directed RNA polymerase.[30,32]

No difference was found between the ability of Zn(II)KTS and $ZnCl_2$ to inhibit either glycolysis or the formation of protein; however, Zn(II)KTS was slightly more active than $ZnCl_2$ as an inhibitor of respiration. The sensitivity of respiration to zinc ions has been earlier documented by a number of laboratories.[33–36] Although the respiratory processes of sarcoma 180 ascites cells are more sensitive to Zn(II)KTS than to $ZnCl_2$, the magnitude of the inhibition does not seem to be sufficient to account for the greater vulnerability of these neoplastic cells to the cytotoxic action of the zinc chelate. Thus, the precise biochemical action of Zn(II)KTS, responsible for the cytotoxicity of this agent, would appear to be subtle and non-detectable by the techniques employed in this study.

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