Metal Chelates of Glyoxal and Methylglyoxal Bis(guanylhydrazones)

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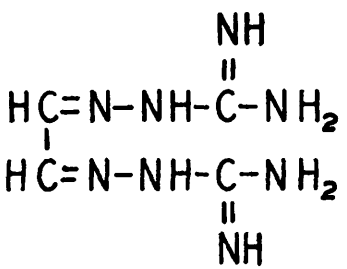
In 1958 Freelander and French described for the first time the antitumor activities of glyoxal bis(guanylhydrazone) (GAG), I, and methylglyoxal bis(guanylhydrazone) (or Methyl-GAG), II, in mice and



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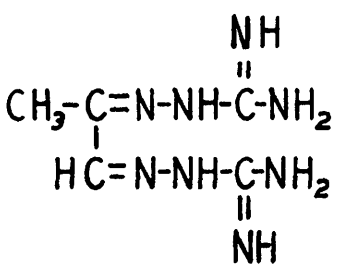

II

rats. These compounds, administered as the diacetates in the diet, lengthened the life of mice bearing L1210 leukemia by 135% and 72% respectively. The compounds are salt-forming bases; they are stable in acid media, but less so in alkaline media.

Methyl-GAG has been used against at least one form of human leukemia. Infused intravenously at the rate of 1 mg/kg day, the compound gave complete remissions for 9 of 13 patients. However the therapeutic dose is close to the toxic level (Freireich, Frei, and Karon, 1962).

Methyl-GAG has a methyl group and GAG does not. The presence of this methyl group no doubt produces small differences in solubilities, steric effects, etc. which lead to differences in ease of transport, adsorption on constituents of various tissues, etc. and ultimately to differences in biological activities.

The purpose of this work was to determine whether the biological difference between GAG and Methyl-GAG could be due to their abilities to complex metals. Both compounds are known to form complexes with

cobalt(II), nickel(II), and copper (II) ions (Donovan and Bailar, 1964). GAG forms 1:1 complexes which apparently are polymeric in nature, being highly insoluble in water. Methyl-GAG also forms 1:1 complexes but these do not precipitate from water solution and may be unstable therein. However the stability constants of these complexes were not measured, and the extent of complexation under biological conditions was unknown.

Consequently the stability constants of the complexes of 11 metals with both GAG and Methyl-GAG were measured in aqueous solution by the potentiometric method (Albert and Serjeant, 1962a). The results of these determinations are listed in Table I. Precipitation of the lead complex prevented the determination of its stability constant. Oxidation of the iron(II) complex occurred even in a nitrogen atmosphere. These results agree with the sequence of stability constants of complexes of other ligands with the same metals with the exception of cobalt(II) and manganese(II). Mellor and Maley (1947, 1948) found the stabilities of bivalent cation complexes as:

$$Pd > Cu > Ni > Pb > Co > Zn > Cd > Fe > Mn > Mg$$

The magnitudes of these stability constants do not necessarily represent the amount of complexation occurring in a biological medium where the pH is quite constant and the concentrations of metal ions vary widely. Consequently the concentrations of complexes hypothetically formed in human blood serum between the most common metals present (Altman, 1961) and GAG or Methyl-GAG were calculated by means of a computer program (Perrin and Sayce, 1967). The concentrations of GAG and Methyl-GAG used for these calculations are representative of those which

TABLE I. STABILITY CONSTANTS OF COMPLEXES

Methyl-GAG: pK_{a1}, 7.56; pK_{a2}, 9.20
GAG: pK_{a1}, 7.14; pK_{a2}, 8.68

Metal	Log K	
	Methyl-GAG	GAG
Cadmium(II)	4.95	4.96
Cobalt(II)	6.17	5.82
Copper(II)	7.82	6.88
Magnesium(II)	5.00	4.86
Manganese(II)	5.28	5.28
Nickel(II)	5.26	4.99
Strontium(II)	4.98	4.90
Zinc(II)	5.28	5.02
Aluminum(III)	11.22	10.40
Chromium(III)	12.36	11.18
Iron(III)	15.60	14.64

may be in the blood serum of patients treated with Methyl-GAG (Freireich, Frei, and Karon, 1962). Since human blood serum contains many substances, both organic and inorganic, capable of complexing metals (Altman, 1961), these were included in the calculations also insofar as possible to a total of 69 complexes. Concentrations of these 69 complexes, including those of GAG or Methyl-GAG, were calculated at pH 7.4 by an IBM 7040 computer. The results for the GAG and Methyl-GAG complexes appear in Table II.

TABLE II. PERCENTAGE OF METALS IN HUMAN BLOOD SERUM COMPLEXED BY GAG AND METHYL-GAG AT PH 7.4.

Compound	Concn. of Compd., $10^{-6}M$	Mg	Zn	Fe	Cu	Mn	Co
GAG	6.2	0.0045	0.0113	9.04	0.0038	0.018	0.0091
Methyl-GAG	6.2	0.0024	0.0096	1.05	0.0163	0.0096	0.0049
GAG	12.5	0.0093	0.0235	17.0	0.0079	0.0372	0.0189
Methyl-GAG	12.5	0.0048	0.0194	2.10	0.0328	0.0194	0.0098
GAG	25.0	0.0194	0.0488	30.0	0.0165	0.0773	0.0393
Methyl-GAG	25.0	0.0097	0.0387	4.1	0.0657	0.0389	0.0197
GAG	50.0	0.0412	0.103	47.6	0.0350	0.164	0.0835
Methyl-GAG	50.0	0.0195	0.0777	7.9	0.132	0.078	0.0395

TABLE III. RATIOS OF METALS COMPLEXED BY METHYL-GAG AND GAG

Concn. of GAG or Methyl-GAG, $10^{-6}M$	Mg	Zn	Fe	Cu	Mn	Co
6.2	0.535	0.848	0.116	4.25	0.536	0.537
12.5	0.522	0.826	0.123	4.16	0.522	0.523
25.0	0.503	0.795	0.137	3.98	0.504	0.503
50.0	0.475	0.751	0.167	3.77	0.475	0.473

Table III shows the ratios of metals complexed by Methyl-GAG and GAG.

The percentages of metals in human blood serum complexed by GAG and Methyl-GAG at low concentrations at pH 7.4 are generally quite small as shown in Table II. However these compounds even at low concentrations are able to complex appreciable amounts of the total iron in the serum. GAG complexes six to nine times more iron than Methyl-GAG under these conditions as shown in Table III. For most of the other metals the Methyl-GAG complexes are only one-half as abundant as the GAG complexes under the same conditions. Copper however is a striking exception in this regard since its complex with Methyl-GAG is four times as abundant as its complex with GAG under the same conditions.

The toxicity of these compounds may be due to their tendency to complex iron. The fact that GAG complexes iron under biological condi-

tions more readily than Methyl-GAG does may account for the preference for Methyl-GAG in the treatment of human leukemia. The fact that Methyl-GAG chelates copper better than GAG does may indicate a possible synergistic effect of copper and Methyl-GAG. The active agent may be the copper complex rather than the copper or the Methyl-GAG. The presence of added copper ions increases the antitumor effect of pyruvaldehyde bis(thiosemicarbazone) (Cappuccino, 1966) and 3-ethoxy-2-oxobutyraldehyde (Ketoxal) bis(thiosemicarbazone) (Booth and Sartorelli, 1966). The synergistic effect of copper with Methyl-GAG and other agents is now being studied in this laboratory.

EXPERIMENTAL

Materials—GAG and Methyl-GAG were obtained from Nutritional Biochemicals Corporation, Cleveland, Ohio (for chemical and investigational use only), both as the dihydrochlorides. The metal perchlorates were obtained from G. Frederick Smith Chemical Company, Columbus, Ohio. These salts were dissolved in carbon dioxide-free water and the solutions were assayed for the metal ions by use of ion-exchange columns.

Determination of ionization constants—Ionization constants were determined by titrating weighed amounts of the two salts with 0.0273 *N* carbonate-free potassium hydroxide at a temperature of 25 C with a Research Model Beckman pH meter (Albert and Serjeant, 1962b). The results shown in Table I were reproducible to the values shown.

Determination of stability constants—Stability constants were also determined by the potentiometric method (Albert and Serjeant, 1962a). In this case the metal perchlorate and the GAG or Methyl-GAG salt were dissolved in water and the pH determined at intervals as carbonate-free potassium hydroxide was added. In some cases the determination was interrupted by the appearance of a precipitate which made further measurements meaningless. Calculations of the stability constants were made by means of an IBM 7040 computer.

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