THE METABOLIC FATE OF 6-MERCAPTOPURINE AND ITS BISMUTH AND PALLADIUM COMPLEXES

by

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ABSTRACT

This research proposal was presented in order to determine the metabolic fate of 6-mercaptopurine (6-MP) and its bismuth and palladium complexes. This was done by manner of determining the levels of the metals, bismuth and palladium, in the blood serum of rats that were intraperitoneally administered 6-MP, the bismuth complex of 6-MP, and the palladium complex of 6-MP. Two conclusions were made as a result of this study: (1) free 6-MP proved to be more toxic than either of the metallo-complexes, and (2) the levels of bismuth and palladium in all three treatment groups were no different from those in the control group indicating that the compounds' greatest therapeutic effects are not in the circulatory system of the mammals.

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INTRODUCTION

The original objective of this research proposal was to determine the levels of the metals palladium (Pd) and bismuth (Bi) in the various organs and body fluids of rats that had been administered 6-mercaptopurine (6-MP), the palladium complex of 6-MP, and the bismuth complex of 6-MP. It was also suggested at one point that the levels of uric acid and inorganic sulfate be determined in the urine and feces of the rats. However, due to the lack of equipment necessary for collecting the urine and feces, this latter part of the proposal was deemed improbable.

Because of several problems, the original objectives were not totally completed. In fact, only one aspect of the objectives was completed--that being the determination of the palladium and bismuth levels in the blood serum of the rats. Five organs, as well as the blood, were extracted from every rat--the heart, lungs, kidneys, spleen, and liver. The organs were frozen for analysis at a later time, but, due to unforseen circumstances, were not analyzed. A discussion of the problems encountered shall be presented later in this report.

REVIEW OF THE LITERATURE

Any form of cancer is characterized by the excess and rapid production of malignant cells. The excess production and accumulation of leukocytes are involved in leukemia, cancer of the blood. Several investigations have already been conducted on the immunosuppressive and possible chemotherapeutic activity of 6-MP and its platinum and palladium complexes in the rat and the chick (8,9,10,13). There has not been as much work done

on the bismuth complex of 6-MP as has been done on the platinum and palladium complexes and free 6-MP. In better understanding the activities of these drugs it will be necessary to trace their metabolic fates.

Six-mercaptopurine has been found to have anticancer properties.(18) Many metallo-purine complexes using 6-MP as the purine ligand have been synthesized. The logic behind this type of inorganic complex is two-fold as postulated by Stanley Kirschner and his associates(5). The coordination of metal ions to viral proteins believed to be involved in carcinogenic tissues releases the carcinostatic ligand (6-MP) to further inhibit abnormal growth, and, thus, provide a possible enhancement of anticancer activity over the corresponding ligand alone.

It has been suggested that there may be some correlation (a) between antileukemic effects and (b) between toxicity and immunosuppression threshold of these compounds (10).

Six-mercaptopurine has been studied in some detail. However, the details of its mechanism and loci of action and of its metabolic interconversions are not yet completely understood. A few suggestions of how 6-MP works include: (1) that it works via inhibition of glycolysis(7), (2) that it works through interference with biosynthesis of purine-containing coenzymes (1) and, (3) the most accepted--its immunosuppressive and anticarcinogenic effects are considered to be mainly through its ability to interfere with nucleic acid biosynthesis (15), which is depicted in Figure 1. Figure 2 shows how 6-MP affects nucleic acid biosynthesis through its metabolites. If this basic mechanism of action is correct, it follows that this drug should block those lymphocyte functions which are dependent on cell division. Comparing Figure 1 and Figure 2, one can see the many sites of interference 6-MP and its metabolites impose on normal purine metabolism.

Normal regulation of purine metabolism depends largely upon a delicate balance of adenine and guanine nucleotides. While ATP is required for GMP synthesis, and GTP is required for AMP synthesis, the inhibitions imposed upon their synthesis from IMP assure a proper balance of these nucleotides. Any interference of this balance by 6-MP or its metabolites may be likely to produce problems in normal purine metabolism.

G. B. Elion considers enzymes involved in transformations of IMP to SAMP and XMP to be especially vulnerable to competetive inhibition by 6-MP metabolites because of the relatively high binding constants of the metabolites and the relatively low natural substrate concentrations.

The thioinosine nucleotides can be seen to interfere at many levels of nucleic acid biosynthesis. The extent to which and the mechanisms by which the thioxanthylic and thioguanylic nucleotides interfere, however, is still not fully clear. Whether or not these compounds successfully inhibit the nucleic acid biosynthetic enzymes is not fully understood either (3). There is still the very likely possibility that they may have their major effect by incorporation into nucleic acids. These may then act in disrupting normal cell metabolism by causing chromosomal breaks, initiating formation of nonfunctional proteins, or inhibiting cell division or differentiation. The mechanism of this antitumor agent in effect interferes with the replication of the cell (see Figure 3).

A major problem in the therapeutic use of 6-MP is its toxicity. Already high uric acid levels in leukemia patients are further increased by treatment with 6-MP (6) because of the fact that 6-MP may be incorporated into nucleic acids, and nucleic acids when degraded will yield uric acid as one of their products.

Mammals dispose of most of their waste nitrogen in the form of urea by way of the urea cycle. Six- mercaptpurine is a nitrogen-containing

purine. Excess nitrogen in the blood that cannot be accomodated by the urea cycle will cause a rise in the pH of the blood and may kill the mammal if present in great excess.

A possible answer to the toxicity problems may lie in the use of the metallo-complexes of 6-MP (see Figure 4). A dual carcinostatic action for these compounds has been suggested(5). Viruses are thought to be involved in many forms of cancer. Coordination of the metal ions of the complexes with the viral protein is one form of action; release of the 6-MP carcinostatic ligand enhances the effect. It has also been suggested that the sulfhydryl group site of used in complexing of 6-MP with the metal may be the mechanism for reduction of toxicity seen with the use of metallo-complexes (17). During degradation of monosubstituted thioureas, the formation of H_2S has been blamed for toxic effects. However, the complexing of the S-H group may be expected to reduce toxicity without impeding the therapeutic function of 6-MP.

EXPERIMENTAL

The following procedure was used for the basis of this research proposal:

1. Inject the rats intraperitoneally with 50 mg/kg/day for 3 days with the appropriate drug. Sacrifice five rats from each group on the fourth day, and the other five from each group on the seventh day.

Group	#per group	Treatment	Average Weight/Rat	Average Dose/Rat/Day
1	5	3-day Control*	558.2 gms	-----
2	5	7-day Control	572.2	-----
3	5	3-day 6-MP*	483.8	24.19 mg/kg
4	5	7-day 6-MP	564.0	28.20
5	5	3-day Bi 6-MP*	570.8	28.54
6	5	7-day Bi 6-MP	547.6	27.38
7	5	3-day Pd 6-MP*	571.2	28.56
8	5	7-day Pd 6-MP	470.4	23.52

*sacrifice on day #four.

2. Exsanguinate each individual rat into a heparinized funnel and test tube. Centrifuge for 20 minutes at 1200 rpm and remove the serum. Dilute the serum 1:3 with distilled water and determine the levels of palladium and bismuth using the atomic absorption spectrophotometer.

3. From each individual rat remove the following organs: the heart, lungs, kidneys, spleen, and liver. Ash the organs following the procedure outlined in Figure 6, and determine the levels of palladium and bismuth using the atomic absorption spectrophotometer.

CONCLUSIONS

The only portion of the rats that was analyzed for the levels of bismuth and palladium was the blood serum. The organs were not analyzed due to various elements. Perhaps the most significant conflict was disorientation, unorganization, and lack of equipment. I had neglected to make certain that our laboratory had sufficient crucibles for the ashing procedure. There were none available and, hence, had to be ordered. They arrived too late for any of the organs to be analyzed.

Another setback was the fact that the bismuth lamp used in the atomic absorption spectrophotometer was not functioning, so, a new one had to be ordered and took several weeks to arrive as well.

However, there are two conclusions that can be made from the work done here. First, the free 6-MP proved to be more toxic than either of the metallo-complexes, thus, supporting earlier results from other people's work along these lines. In the group sacrificed on the fourth day, two rats of the 6-MP treatment group were dead even before they were sacrificed. In the group sacrificed on the seventh day, four of the five rats of the 6-MP treatment group died before being sacrificed, whereas none of the other treatment groups lost any members.

Secondly, the levels of bismuth and palladium in all three treatment groups were no different from those in the control group. This tends to

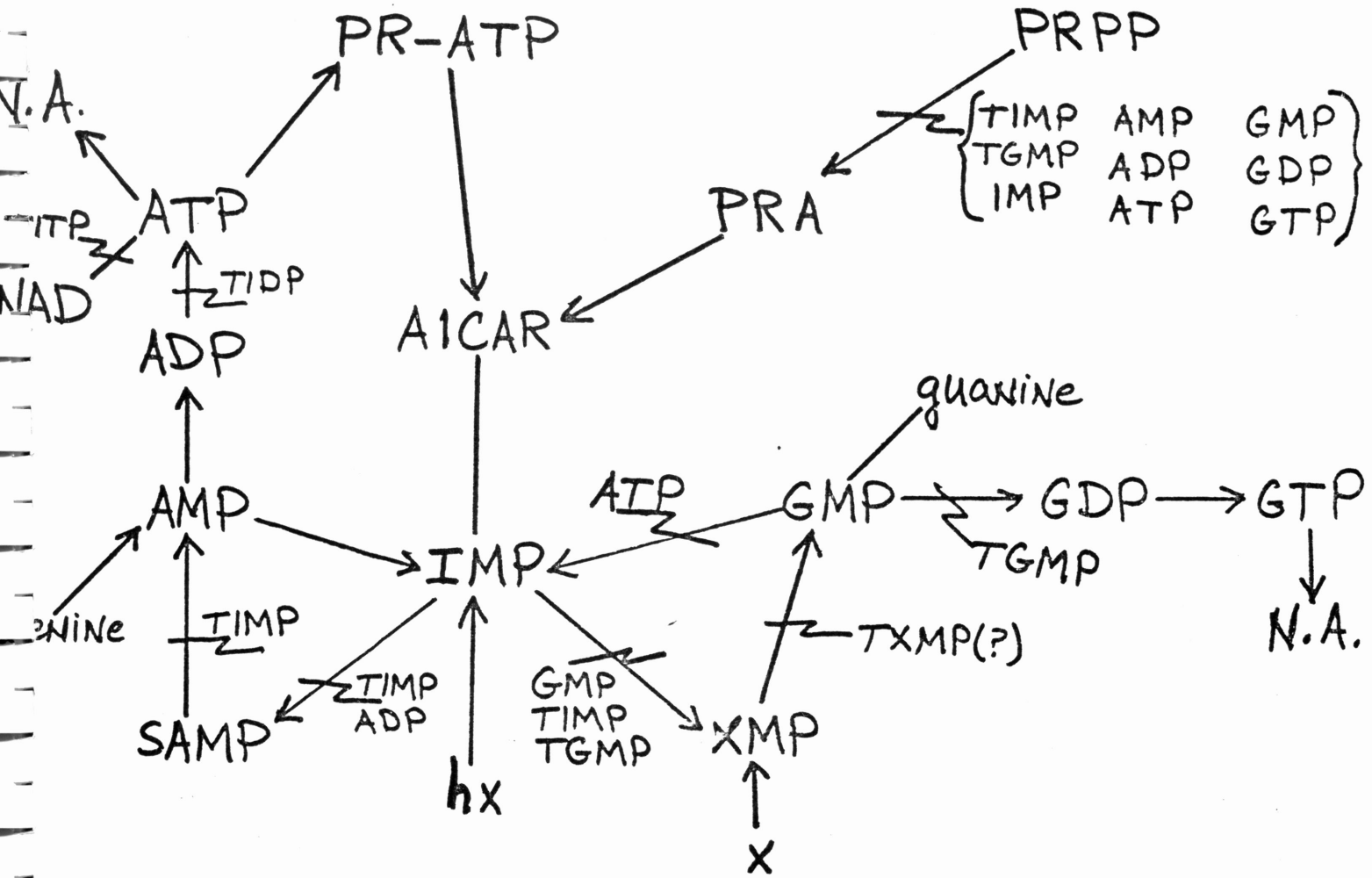
indicate that neither 6-MP, Bi 6-MP, nor Pd 6-MP present their greatest therapeutic effects in the blood of the mammals. It would appear that free 6-MP and its metallo-complexes would present their greatest therapeutic effects in some organ of the body--quite possibly one of the five organs extracted but which were not analyzed.

TABLE OF RESULTS

	Fourth-day Sacrificing			Seventh-day Sacrificing		
		Bi	Pd		Bi	Pd
Control						
1		@	@		@	@
2		@	@		@	@
3		@	@		@	@
4		@	@		@	@
5		@	@		@	@
Free 6-MP						
1		@	@		@	@
2		@	@	*		
3		@	@	*		
4	*			*		
5	*			*		
Bi 6-MP						
1		@	@		@	@
2		@	@		@	@
3		@	@		@	@
4		@	@		@	@
5	*				@	@
Pd 6-MP						
1		@	@		@	@
2		@	@		@	@
3		@	@		@	@
4		@	@		@	@
5		@	@		@	@

* dead before they were sacrificed but not used in the experiment
@ levels of the metals identical to those of the control groups, which were significantly less than one part per million.

Purine Metabolism Figure 1



6-MP Metabolism Figure 2

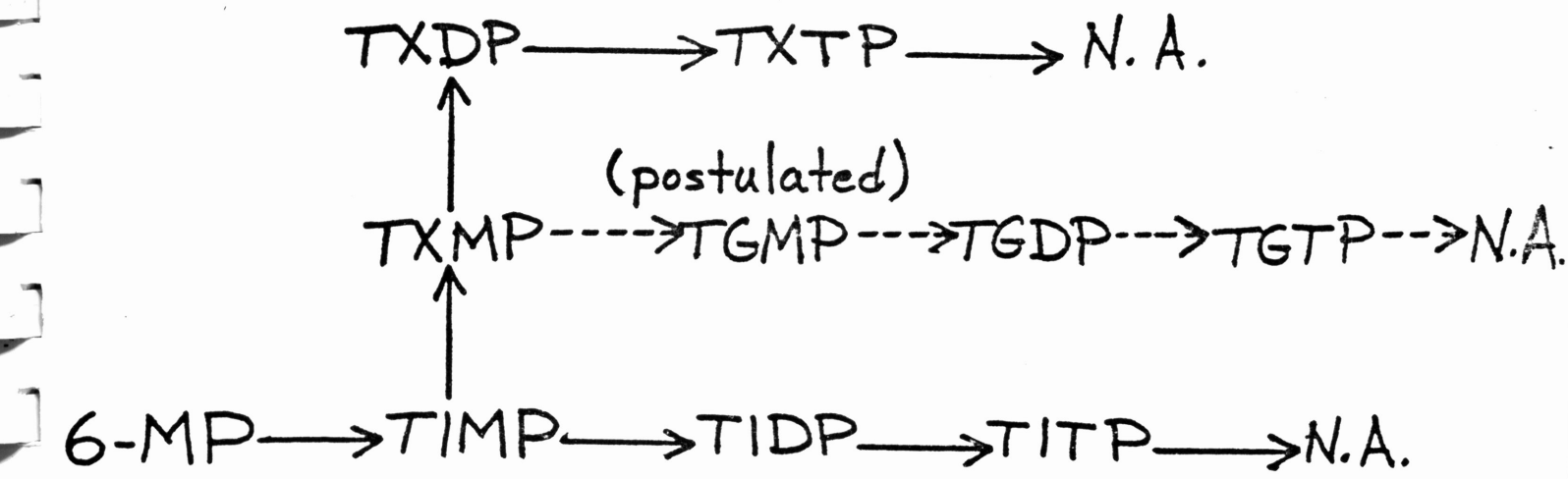
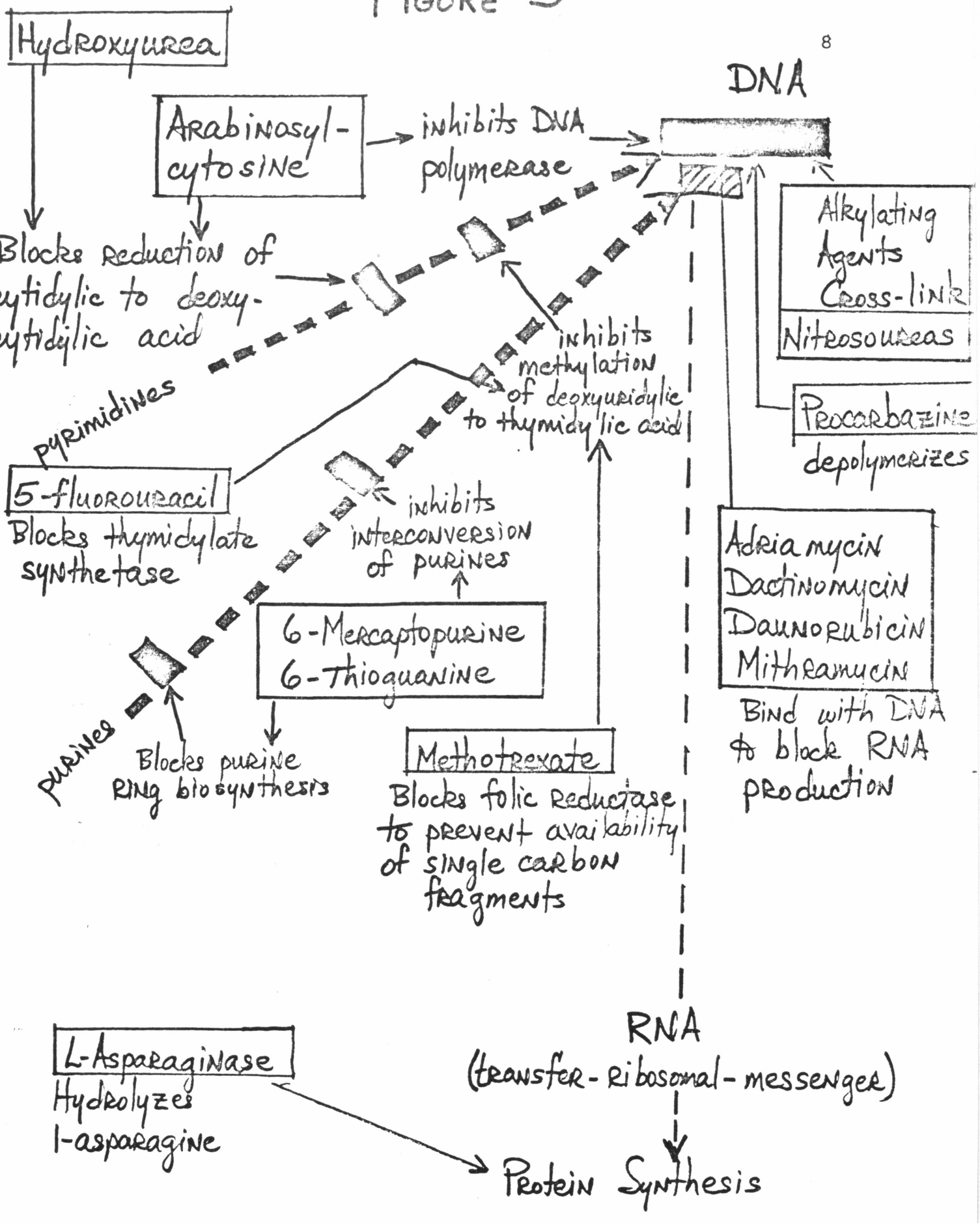


FIGURE 3



The mechanism of action of several antitumor agents that interfere with the replication of cells.

FIGURE 4

MP Platinum complex

6-MP Pt complex synthesized by KIRSHNER and found by the NIH to possess anticancer activity against Sarcoma 180 in Swiss mice and adenocarcinoma in BDF mice.
Less acute toxicity than 6-MP alone and demonstrated by lower mortality and less weight loss.
Permanent tolerance of skin homografts in young chicks.
Leukopenia induction with more rapid recovery than 6-MP. (leukocyte)

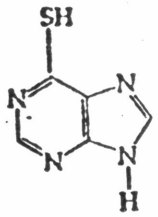
MP Pd Complex

Synthesized by KIRSHNER and found by NIH to possess anticancer activity against Sarcoma 180
Less acute toxicity than 6-MP alone.
Leukopenia induction with more rapid leukocyte recovery than 6-MP alone.
Substantial reduction in sera uric acid level over 6-MP.
Substantial reduction in liver uric acid levels over 6-MP.

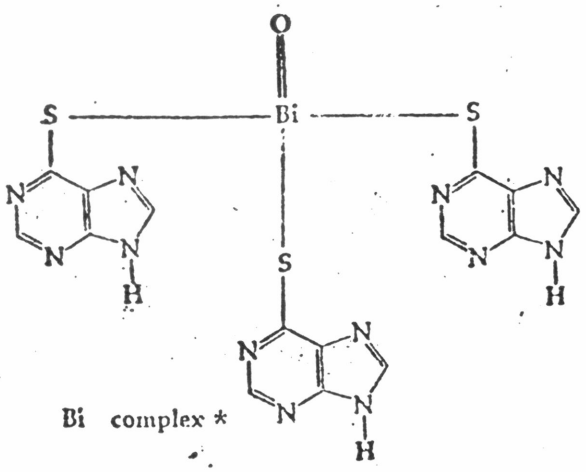
MP Bi Complex

Synthesized by KIRSHNER and found by NIH to possess anticancer activity against Sarcoma 180.
Less acute toxicity than 6-MP alone.
Physiological concentration of adenine metabolites higher in tissues samples taken 24 hours after administration than at 3 hours after.
Kidney conc. > Serum > Liver

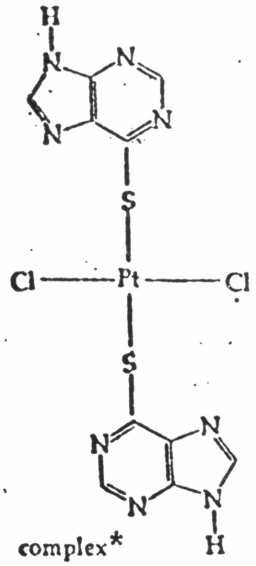
FIGURE 5



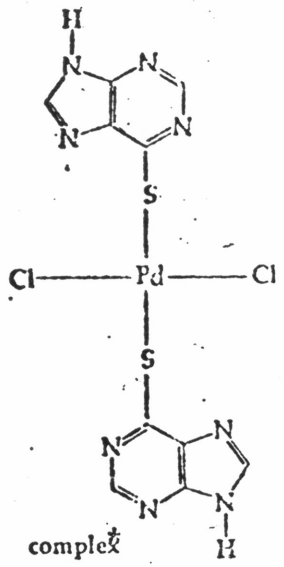
6-Mercaptopurine



Bi complex *



Pt complex*



Pd complex*

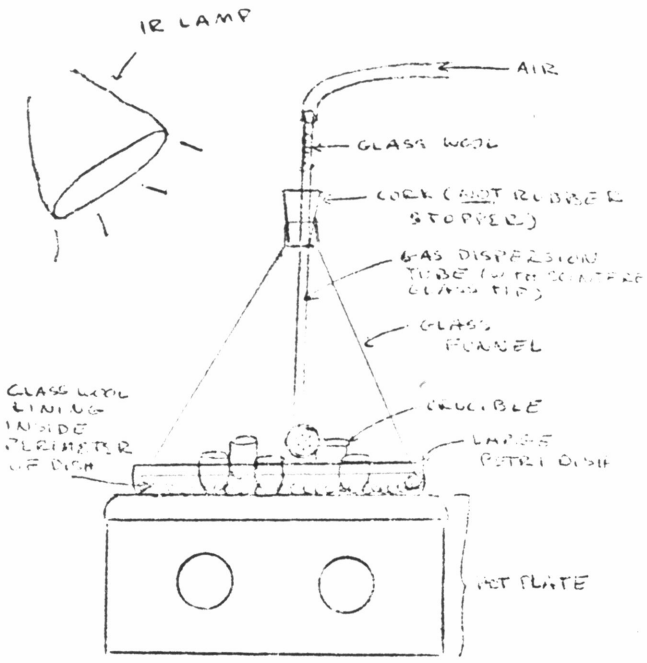
*Postulated structure based on Kirschner, et al., J. Med. Chem. 9, 369-372, 1966.

FIGURE 6

EXTRACTION OF METALS FROM BIOLOGICAL SUBSTANCES

This is the way to do it according to:

Thiers, R.A., Meth. Bioch. Anal. - 5 - 273-335, 1957.



Dessication

1. Place sample in dessication apparatus in the hood.

2. For liver and other large samples, pre-weigh vessel containing the tissue , dessicate bit by bit, weigh the empty vessel and calculate the total wet weight of the tissue.

3. Keep in dessication apparatus for 15 min. to several hours, increasing heat till tissue is charred.

4. Turn off the hotplate, leaving petri dish there to cool - otherwise it will crack.

Ashing

1. Cover crucible and place in muffle furnace at 250°C

2. Raise to 450°C withing an hour (in 50° steps) and leave till ashed.

3. Check at 24 hours. If any black carbon remains, add about 1 ml concentrated HNO_3 to dissolve contents of dish. Then evaporate under dessication apparatus. Replace in muffle furnace.

4. Check 24 hours later.

   a) If not ashed, dissolve what you can in minimal volume of 6M HCl and pipette carefully away from carbon residue. Repeat this wash and combine washings. Dry carbon residue under dessication apparatus (or IR lamp alone). Add a few drops concentrated HNO_3 and evaporate. Place about 2 hours in muffle furnace. This should do it. No carbon should remain. Return washings to crucible to dissolve remaining sample.

   b) If ashed, dissolve in minimal amount 6 M HCl.

5. Heat to aid dissolution of ash which is generally complete if enough acid is used and enough time allowed and the silica content is not too high.

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