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6-MERCAPTOPURINE, A NEW ANTI-METABOLITE IN LEUKEMIAS

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Submitted in Partial Fulfillment for the Degree of Doctor of Medicine

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I

INTRODUCTION

Leukemias: Clinical and Pathological Course

The fatal disease known as Leukemia was first observed by Barth, although the initial examination of leukemic blood was made by Donne in 1839. Its first description as a clinical entity was made independently by Bennett and Virchow in 1845. In the course of a little over a century, treatment of this disease has undergone a radical change. One of the newest drugs to show promise in the treatment of Leukemia is 6-Mercapto-purine, one of the new purine antagonists. The purpose of this monograph is to present a comprehensive review of the mechanism of action of this new anti-metabolite, and to summarize the recent experimental and clinical data available on this drug.

Leukemia, as it is well known, is primarily a disease of the blood and blood forming organs. Burchenal (1). The normal marrow is made up of three separate components: the myeloid elements which produce the polymorphonuclear leukocytes of the peripheral blood; the erythroid elements which produce the red cells and hemoglobin; and the megakaryocytes which produce platelets. In

leukemia, the marrow is invaded or replaced by abnormal neoplastic cells, which may arise from any cell type, with replacement of the normal elements. This results in a lack of the three normal types of cells in the blood which in turn can produce various symptoms of the disease. Lack of polymorphonuclear leukocytes leads to increased susceptibility to infection, particularly by the pyogenic organisms. Lack of adequate red cells diminishes oxygen transport, and produces symptoms of anemia such as lassitude, easy fatigability, and dyspnea on exertion. Deficiency of platelets causes a hemorrhagic diathesis with petechiae, ecchymoses, epistaxes, and the various symptoms of cerebral, gastrointestinal, and urinary bleeding. The ability of the leukemic cells to invade tissue leads to splenomegaly, hepatomegaly, lymphadenopathy, and osseous involvement with consequent pain in bones and joints. There may also be localized tumor masses and skin infiltrations. However, the signs and symptoms in any particular case depend upon the type of leukemia. In the chronic granulocytic form, for example, where the peripheral blood has a high percentage of myelocytes and mature polymorphonuclear leukocytes, the symptoms of infection are uncommon. The replacement of erythroid elements of the marrow leads to anemia, and infiltration of the myelocytes into the spleen and liver results in their respective

enlargement.

However, in chronic lymphocytic leukemia, the predominant signs are usually infiltration and enlargement of lymph nodes and often splenomegaly. In this form, skin infiltrations are more common, and an increase tendency to petechiae and ecchymoses frequently occur.

In acute leukemia, the manifestations of a severe deficiency of all normal formed elements are most apparent. Lack of resistance to infection, severe anemias, severe hemorrhagic diathesis, ulcerations of mucosa and gums, and infiltrations of spleen, nodes, liver, and other tissues are common.

Methods of Treatment of Leukemia

The treatment of leukemia has been in the past, and is yet, one of palliation and supportive therapy, rather than a treatment correcting or curing the condition. Arsenic was one of the first chemotherapeutic agents available for the treatment of chronic granulocytic leukemia, being employed by Lissauer in 1865. Burchenal (2). This compound will produce definite remissions in early stages of chronic granulocytic leukemia, but the administration is often accompanied by nausea and vomiting.

Other forms of treatment used in chronic granulocytic leukemia have included radiation therapy, urethane, nitrogen mustard, TEM, and Myleran. Radiation therapy has been given as localized X-ray, total body spray, or IV or oral radioactive phosphorus. Burchenal (2) feels that radiation therapy is still the treatment of choice in chronic myelocytic leukemia, if the proper facilities are available.

It has been shown that in acute leukemias, irradiation is contraindicated, but that the antimetabolites and the hormones are of value. It has been proposed by Tivey (3) that there is a relation between so-called spontaneous remissions and those induced by therapy. In a series of 76 "spontaneous" remissions, 38 were preceded by a febrile illness. Other remissions were preceded by injection of foreign proteins, Miller (4), or nonspecific trauma such as surgery and eclampsia. Tivey (3). Tivey believes that there is one common denominator in the above mentioned cases of spontaneous remissions. That factor is adrenal stress. There have been spontaneous remissions recorded in 22 per cent of a series by Bessis and Dausset,(5) in which the remission followed a blood transfusion. Tivey believes that the cause of these remissions may be a direct transfer

of ACTH and/or "cortical Steroids," or a stimulus for the production of these hormones by the patient. Although the evidence in favor of this theory is by no means conclusive, the similarities in character and duration of the remission are suggestive. According to Tivey's statistics, the differences in median duration of remission shown between cortisone and "spontaneous" or transfusion remissions could occur by chance alone in at least 15 per cent of the cases.

It was not until the anti-metabolites were successfully used in the treatment of acute leukemia that significant advances were made in the treatment of this disease. This was first accomplished by Farber (6) in 1948, when he showed that the anti-metabolite, 4-aminopteroylglutamic acid, was effective in the chemotherapy of leukemia. As is well known, an anti-metabolite is a compound whose chemical structure differs only slightly from some normal metabolite, so that the anti-metabolite is able to enter into the same enzyme systems as the normal metabolite, thus blocking the enzyme system. Folic acid is a vitamin necessary for the growth and maturation of normal erythroid and myeloid tissue of bone marrow, as well as being necessary for the growth and maturation of almost all living things. The anti-metabolite, 4-aminopteroylglutamic acid (Aminopterin),

is almost structurally identical to the chemical formula of folic acid. Thus, by competitive inhibition, maturation of cells is prevented. It is believed that neoplastic cells are affected more than normal cells because of their much more rapid rate of cell division.

Another group of drugs which has been used in the treatment of the leukemias may be loosely classified as the cytotoxins. This includes nitrogen mustard and synthesized products closely resembling the action of this chemical, such as triethylene melamine, triethylene phosphoramide, and one of the newest drugs in this category, triethylene thiophosphoramide, known as Thio-TEPA. It is believed that these drugs are actual cytotoxins, acting directly on the chromosomes, especially when cells are in the dividing phase. This results in an overall bone marrow depression. Shay et al.(7).

Another drug, Myleran, is a sulfonic acid ester which, in chronic granulocytic leukemia, selectively depresses myelopoiesis, but not the formation of lymphocytes or platelets. It is supposedly more selective than nitrogen mustard or the folic acid antagonists.

Urethane, an ethyl ester of carbamic acid, has also been used in chronic leukemias. It acts as a selective metabolic inhibitor for white cells. Leukemic

cells are much more susceptible than normal white cells, and the most susceptible are the early myeloid cells.

II

MECHANISM OF ACTION OF 6-MERCAPTOPURINE IN LEUKEMIAS

In order to understand more fully the specific action of 6-MP in leukemias, it is necessary to understand the biosynthesis of nucleic acids. Nucleic acids, combined with simple proteins of basic character, make up a large part of the nuclear material of all living cells, and are also present in the cytoplasm. West and Todd (8).

Davidson (9) believes that the nucleic acids are essential for all such self-reproducing units as the gene, and that they are abundant in all cells in which a rapid protein synthesis is taking place either for the reproduction of the gene proteins in the chromosomes, or in the production of the main part of cytoplasmic protein for growth or for secretion.

Hydrolysis of a nucleoprotein results in the following: White (10)

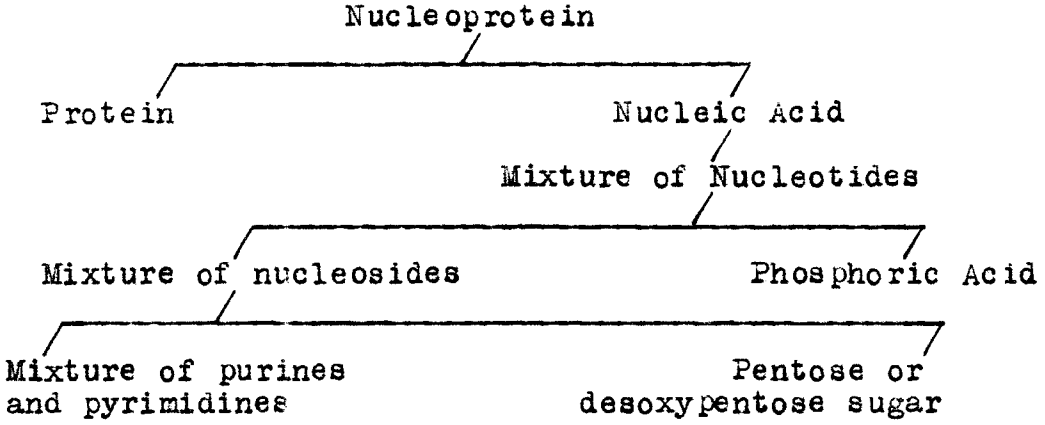


Figure 1

As may be seen in the above diagram (Figure 1), the fundamental units of the nucleic acids are the nucleotides. These are so linked, as is shown in Figure 2, that the phosphate residues of each nucleotide act as bridges in forming diesters, thus forming the polynucleotides.

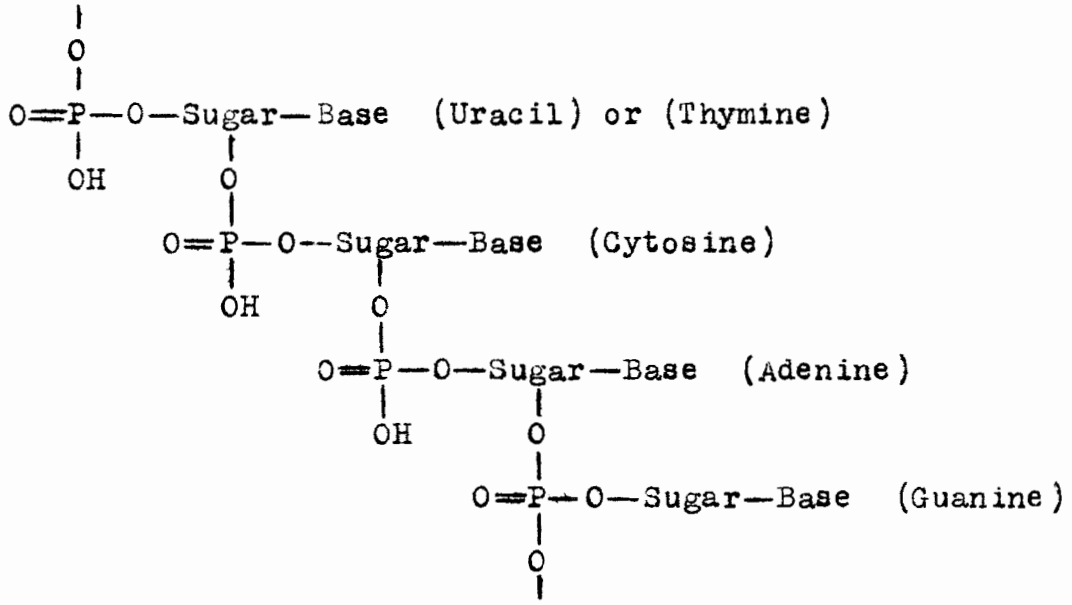


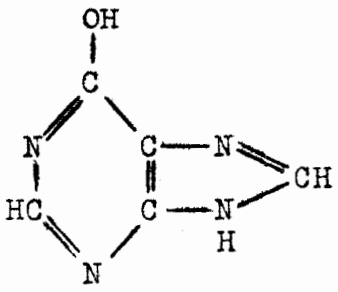
Figure 2

The nucleic acids may be classified into two large groups depending on whether they contain a pentose sugar (ribose) or a desoxypentose sugar (desoxyribose). These two nucleic acids are therefore called ribose nucleic acid (RNA), and desoxyribose nucleic acid (DNA). This could be illustrated in Figure 2 by the substitution of "ribose" or "desoxyribose" for the word "sugar." The two kinds of nucleic acid contain the same purines,

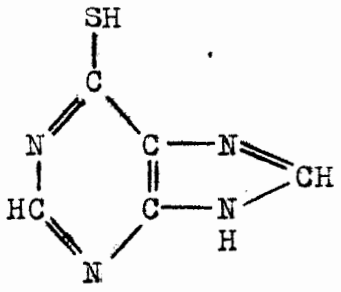
adenine and guanine, but differ in the pyrimidines. On hydrolysis, RNA yields cytosine and uracil, whereas DNA yields cytosine and thymine. This is illustrated in Figure 2 by the substitution of uracil or thymine for the word "base." The other purines and pyrimidines given in parenthesis are those found in nucleic acids. RNA is universally present in the cytoplasm of cells, and recent studies indicate that it may also be present in the nucleus. DNA has not been found in cytoplasm, but is found in the nucleus as parts of the chromosome structure.

The above is a rather brief sketch of the biochemistry of the nucleic acids, and their relation to living cells, normal and neoplastic. Now the question is: "How does 6-MP prevent the formation of nucleic acids, which are so vital to life?" Without a doubt, the explanation here offered is not the final answer. This particular question is surrounded by much controversy, but it is generally believed that the action is one of competitive inhibition. As can be seen in Figure 3, 6-MP is closely analogous in structure to hypoxanthine, adenine, and guanine.

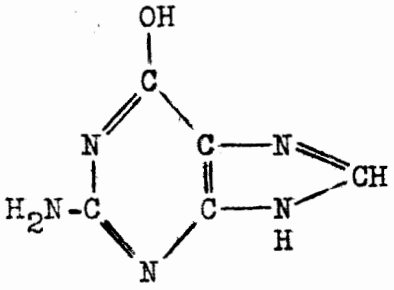
As is seen in Figures 1 and 2, adenine and guanine are essential substances in the synthesis of nucleic acids. It might be logically explained that the



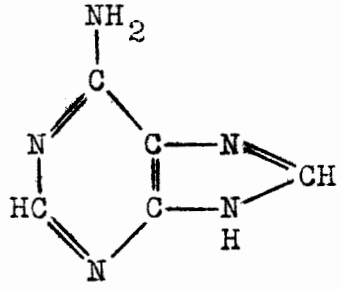
Hypoxanthine



6-Mercaptopurine



Guanine



Adenine

Figure 3

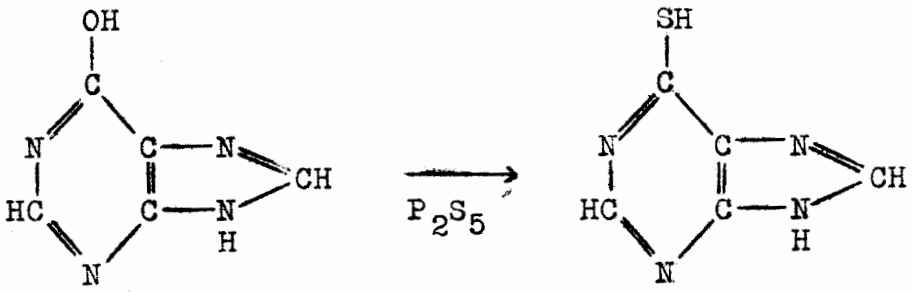
chemical structure of 6-MP so closely resembles the structure of adenine and guanine that it replaces these bases in the formation of the nucleic acids. However, this specifically is not believed to be the case. There apparently may be an interconversion between adenine and guanine which enters into the picture. Brown (11). Furthermore, it is believed that a hypoxanthine-containing metabolite may be necessary in the conversion of adenine to guanine. Skipper (12). Both Skipper and Elion (13) believe, as a result of their experimental work, that 6-MP has its blocking effect upon hypoxanthine, thereby

inhibiting the normal production of adenine, and guanine. It is hoped that further studies will clarify this problem.

III

SYNTHESIS OF 6-MERCAPTOPURINE

Having a basic understanding of the biosynthesis of the nucleic acids, and the mechanism whereby 6-MP prevents their formation, it will now be of interest to consider the basic steps in the development of 6-MP. Hitchings (14) discovered that thiouracil and thiothymine were competitive antagonists of uracil and thymine. Further research led to the development of thioguanine, which proved to be an active antagonist to guanine, thus breaking a link in purine metabolism. Thioguanine was shown to be a purine antagonist, but difficulties in synthesis caused attention to be turned toward the development of 6-MP. Hypoxanthine was treated with phosphorus pentasulfide, producing 6-MP, as is shown in Figure 4.



Hypoxanthine 6-Mercaptopurine

Figure 4

However, the most practical method of synthesis is that developed by Traube. A 4,5,-diaminopyrimidine

is synthesized and, by formylation, converted to a 5-formamido-4-aminopyrimidine, followed by cyclization of the imidazole ring. The compound 4-amino-5-formamido-6-mercaptopyrimidine is cyclized to 6-MP by heating of the sodium salt. This is as seen in Figure 5.

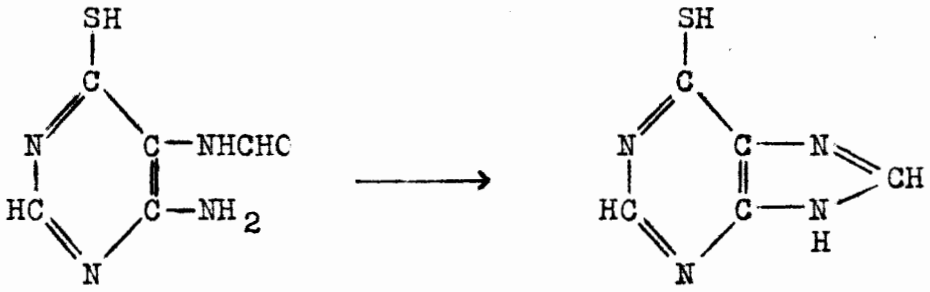


Figure 5

IV

EXPERIMENTAL DATA ON 6-MERCAPTOPURINE

So far, leukemia as a disease has been considered, and a brief review of the various types of therapy has been presented. There has also been a brief presentation on the theory of anti-metabolites and a discussion of the mechanism of nucleic acid biosynthesis. Finally, the theories of the mechanism of action of 6-MP have been reviewed, and a brief report on the synthesis of this new drug has been presented. In order to give the reader of this monographan idea of the type and amount of experimental work which was necessary before this drug could be used in the therapy of leukemia and other neoplastic diseases in humans, a brief review of some of the more significant experimental data on 6-MP will be presented.

Action of 6-Mercaptopurine

In order to study the effects of 6-MP upon living tissue, the Rana pipiens embryo was chosen as a test object by Bieber (15), because it presented a minimum of technical difficulties, and allowed rapid screening with well marked criteria of activity. The developing embryos were placed in test solutions of the various anti-metabolites under consideration, concentrations of the solutions

ranging from 1 to 50 mgm. per cent. Progress was determined by Shumway stages, and comparison with a control group of animals. However, the developing embryo undergoes rapid cellular division and progressive differentiation. It contains many differentiating organs and biochemical systems. In order to minimize the complexity of the resultant relationships, and to provide a system in which a separation of differentiation from cell multiplication could be achieved to some degree, the amphibian's capacity for regeneration of a lost member was utilized. The original paper should be consulted for the details of this study, but basically the program was carried out in this manner. Tails were amputated from tadpoles, and the animals were then placed in the test solutions, and on the seventh day after amputation, measurements of the regenerating blastema length were made by means of an ocular micrometer.

It was thus shown that thioguanine, 8-azaguanine, and 2,6-diaminopurine did not affect development of the embryo at any of the concentrations. Tests determining the activity of 6-MP were made both upon the developing embryos and upon the regenerating tail blastema. It was shown as a result of these experiments that, in both test systems, 6-MP is active primarily against relatively undifferentiated cells.

In order to determine the effects of 6-MP upon the mammalian fetus, Thiersch (16) conducted an interesting series of experiments. His studies were upon the Long-Evans strain of rats, and were conducted in the following manner. After determining the onset of pregnancy by examining the vaginal contents of the animals for sperm, a control group was administered a placebo at the same time as the experimental group received the drug. All rats were sacrificed on the 21st day of gestation, the day prior to expected littering, and examinations of maternal hemoglobin, sternal bone marrow, and uteri were made. The fetuses were counted, measured, and weighed, and inspected for malformation. The head was cross-sectioned to detect internal hydrocephalus. After tabulating the results of several series, it was found that the fetus was most sensitive to the drug at the time of implantation, or within 24 hours of implantation. This corresponds to the seventh and eighth days of gestation. At this time, two doses of 5 mgm./kg. induced death and resorption of half of all fetuses, and two doses of 10 mgm./kg. induced 90 per cent fetal resorptions. Little effect was seen by the drug when administered from the fourth day of gestation onward. However, it was shown that rats treated prior to mating with 3 doses of 30 mgm./kg. produced litters with a small increase in

numbers of stunted and resorbed fetuses. This suggests an adverse effect, possibly that of an incorporation of the drug into the developing ovum.

Biesele (17) conducted a study of the effects of 6-MP on experimental tumors. Combined cultures of mouse sarcoma 180 and mouse embryo skin were found to be differentially inhibited by 6-MP. The procedure was carried on in this manner: Tissue cultures, in a conventional medium of serum, embryo extract, and balanced salt solution, were studied for mitotic activity. Similar cultures of the various tissues were dosed with a fixed amount of the drug under question, and after 24 hours of exposure to the agent, the cultures were fixed in alcohol-acetic acid, and then stained with Feulgen reaction and light green. Counts of mitotic figures and degenerating or pyknotic nuclei were made in 1000 nuclei in the zone of outgrowth in each of several cultures for a given treatment. A tabulation of this data revealed that 6-MP was capable of suppressing cell division to a variable extent in a number of cell strains. The mitotic inhibition was more pronounced in some neoplastic cell strains than in several of the normal tissues tested.

It was also shown that the mitotic suppression caused by 1.0 mM 6-MP in sarcoma 180 cultures could be

relieved in part by several physiological purines. A preparation of coenzyme A was the most effective material in counteracting the mitotic inhibition. If the active material in this case is actually coenzyme A, it is speculated that 6-MP may exert its action by interfering in 2-carbon transfers, or in the energy metabolism of the citric acid cycle in the tissue cultures.

The effects of 6-MP on experimental tumors was studied by Clarke et al. (18) in a different fashion. His studies were employed on female Swiss albino mice in which uniform pieces of Sarcoma 180 were introduced subcutaneously by trocar into the right axillary region. In this manner, the experimental tumors were living in mammalian tissue rather than in culture media. Daily injections of a suspension of 6-MP were started intraperitoneally either 24 or 96 hours after tumor implantation, and were continued for seven successive days. The tumors were then measured through the skin with calipers 24 hours after the last dose. Studies of the other purine analogs were conducted in a similar manner.

Clarke was able to demonstrate in his series that 6-MP effectively inhibited growth of sarcoma 180, which resulted in a prolongation of survival time of the host, and, in a significant number of animals, recovery from the tumor. It was demonstrated that 6-MP induced

alterations in S-180 which resulted in the loss of viability of the otherwise readily transplantable tumor. It was also shown that, in certain instances, S-180 could become resistant to both the inhibitory and oncolytic effects of 6-MP. A treated tumor, which grew following implantation into a normal host, was treated through successive transplant generations with low doses of 6-MP. After two generations, its growth was found resistant to higher doses of 6-MP, and it was found to have 100 per cent "takes" when implanted in normal mice. Microscopic study revealed that the resistant tumor exhibited cyto-plasmic vacuolization, whereas the sensitive tumor did not.

Skipper (19) studied the effects of 6-MP against the solid experimental tumors, adenocarcinomas 755 and Eo 771 and sarcoma 180. The experiments were carried out on mice in a similar manner to that of Clarke's. It was found that 6-MP is greatly inhibitory to adenocarcinoma 755 and moderately inhibitory toward adenocarcinoma Eo 771 and sarcoma 180. Skipper also demonstrated that combinations of 6-MP and azaserine, or 6-MP and A-Methopterin were synergistic in antileukemic activity in L 1210 leukemia in mice. However, in mice with A-Methopterin-dependent lines of leukemia, there was no increase in life span under treatment with 6-MP.

Gellhorn et al.(20), from the Institute of Cancer Research of Columbia University, worked with an experimental brain tumor, Glioma 26. He performed his experiments both in vitro and in vivo, in the manner described previously in this paper. His experiments revealed that 6-MP inhibited Glioma 26 in vivo, but not in vitro. This suggests that the antitumor action of 6-MP may be attributable to the metabolism of 6-MP within the body to a pharmacologically active metabolite. Further experiments have been conducted to substantiate this theory, but nothing conclusive has been reached.

Law et al.(21) studied the effects of 6-MP on experimental lymphocytic leukemias transplanted into mice in a similar fashion to that of Clarke. He demonstrated that 6-MP inhibited leukemic cell growth of leukemia L1210 and several other acute lymphocytic leukemias in a definite, regular, and reproducible manner. Law was also able to develop a resistant subline of leukemia, which would grow optimally either in the presence or absence of the antagonist. The resistant leukemias were resistant not only to 6-MP, but were found to be resistant to all other purine analogs tested: 8-azaguanine, 8-azaxanthine, 2,6-diaminopurine, purine, thioguanine, and chloropurine. The resistant mutant also showed an increased sensitivity to the folic acid antagonists.

Finally, Law demonstrated that when the folic acid antagonist, A-Methopterin, and 6-MP were used simultaneously, there was a potentiation of the antileukemic activity.

Toxic Effects of 6-Mercaptopurine

In addition to the experimental studies on the effects of 6-MP on various tumors, experimental work has also been carried out to determine the toxic effects of 6-MP on the host. This, in the final analysis, is the limiting factor in the employment of the drug in the treatment of neoplasia. Philips et al.(22) conducted a series of experiments on mice, rats, and dogs in an effort to determine the toxic effects of 6-MP. The most consistent of these effects were damage to bone marrow and intestinal epithelium, disturbances in hepatic function, and the possibility of liver necrosis. Philips compared 6-MP with seven closely related purine analogs and found them to have a number of common pharmacologic properties. However, it was demonstrated that renal insufficiency, which is prominent in animals given adenine or 2-chloroadenine, is not found in the use of 6-MP. The end product of 6-MP metabolism, 6-thiouric acid, does not crystallize in renal tubules.

As has been mentioned above, damage to bone marrow is one of the common properties of the purines. The

fact that clinical diseases of hematopoietic origin are sensitive to bone-marrow depressants suggests a common mechanism of action in normal and malignant cells. However, Philips demonstrated that the various purine analogs are not identical with regard to specificity of action in bone marrow. To illustrate: 6-MP, 2,6-DAP, and 6-chloropurine produced similar lesions in intestinal epithelium when given in doses causing depression of bone marrow; but the effects of thioguanine were largely limited to the bone marrow, and 6-methylpurine appeared to selectively depress erythrogenesis. This demonstrates that minor alterations in the structure of purines can produce unexpected changes in their pharmacologic effects.

Metabolism of 6-Mercaptopurine in Mice

Studies have been undertaken to determine the metabolism of 6-MP in mammals. Elion et al.(23) conducted a series of experiments on mice with the aid of radioactive isotopes of 6-MP. One of the isotopes was labeled in the 8-position of the purine nucleus with C^{14}, the other was labeled in the mercapto group with S^{35}. The experiments were designed to determine the length of time the drug remained in the body, the metabolic products formed, and whether the drug was perferentially concentrated in any particular tissues, and whether the

compound was incorporated into the nucleic acids. The length of time the drug remained in the body was determined by pooling urines from 20 mice and determining the radioactivity at various time intervals. It was found that almost 44 per cent of the isotope was excreted in the first four hours, and at the end of forty-eight hours, over 60 per cent had been excreted.

The determination of metabolic products formed was carried out by paper chromatography. The concentration of radioactivity at certain spots revealed that the main excretory products during the first four hours were 6-MP and 6-thiouric acid.

The concentration of 6-MP in the tissues was determined by cold trichloroacetic acid extracts of the individual organs. It was thus shown that the concentration of radioactive material was highest in the intestine, and lowest in the brain, with blood levels twice as high as those of the intestine.

Studies on the incorporation of 6-MP isotopes into the nucleic acids were accomplished with the determination of the radioactive levels of hot trichloroacetic acid extracts of the tissues. It was demonstrated that within three hours after injection of S^{35}-6-MP, radioactive material had been incorporated into both the RNA and DNA fractions of the pooled tissues.

Metabolism of 6-Mercaptopurine in Man

Very little is known of the actual distribution and metabolism of 6-MP in man. However, Hamilton and Elion (24) treated a child with acute stem-cell leukemia and an adult with chronic granulocytic leukemia with the purine isotope 6-MP- 6-S^{35}. Serial blood, urine, and cerebral spinal fluid samples were taken, and 24-hour specimens of feces were collected at 24 and 48 hours. Extracts of the samples were then dried and the radio-activity determined with a special Geiger-Muller counter. Results, which were similar in both the child and adult, demonstrated a rapid metabolism of the drug. The half-time of 6-MP in the blood was calculated to be 90 minutes. It was also found that significant radioactivity was pres-ent in the cerebral-spinal fluid within five minutes after injection of the drug, and that this level was maintained for at least 24 hours. At the end of 24 hours, about

60 per cent of the administered radioactivity had been recovered in the urine. Calculations of the renal clearance of 6-MP corresponded approximately to the estimated rate of glomerular filtration. This suggests that there is little tubular excretion or reabsorption of 6-MP, but that its excretion in urine is probably determined by glomerular filtration rate. Further data suggest that

it is unlikely that 6-MP is confined to the extra-cellular fluid, but that it is distributed generally throughout the total body water. It was also shown that a negligible amount of radioactive material was excreted in the feces.

Further studies are being conducted at the present time to add to what little is known about the metabolism of 6-MP. Many of the theories expressed in connection with the experimental evidence presented in this paper may be proven to be unsound, or may be modified, as further studies are made.

V

CLINICAL DATA ON 6-MERCAPTOPURINE

Indications for Use

There is a relatively large volume of clinical data on 6-MP available from the larger research centers in the United States and other countries. However, Burchenal et al.(25), of the Chemotherapy Service of the Sloan-Kettering Institute, has done the large share of the initial clinical work with 6-MP. In a series of 269 patients with different forms of leukemia, Burchenal was able to demonstrate its value in acute leukemias of both children and adults, with children obtaining the better response. A high percentage of remissions was also obtained in early chronic granulocytic leukemia, although other forms of therapy may be considered to be just as, or more practical. Burchenal found that 6-MP was without value in chronic lymphocytic leukemias, lymphosarcomas, Hodgkin's disease, sarcomas, and metastatic carcinomas.

Farber (26), in a study of the second largest series, demonstrated the effects of 6-MP on 96 patients at the Children's Cancer Research Foundation in Boston. Sixty of the patients had acute leukemia, of which about 50 per cent responded hematologically to 6-MP. This

response consisted of about one-third complete remissions and two-thirds hematological improvement. In addition, two cases of chronic leukemia showed a temporary hematological improvement.

Hall (27), from Stanford University College of Medicine, in a study of 24 adult patients with acute leukemia who were treated with 6-MP, reported an initial remission rate of 54 per cent. This, in his experience, is a significantly higher remission rate than has been reported previously with hormone or folic acid antagonist therapy. The remissions were partial or complete. Complete remissions lasted for several months and did not require the continued administration of 6-MP. When a relapse occurred, a second remission could sometimes be obtained with 6-MP, but the second remission was usually less complete than the first. Partial remissions were maintained most satisfactorily by continued administration of 6-MP. Hall was also able to demonstrate a good clinical and hematologic remission in four out of a series of five adults with chronic granulocytic leukemia undergoing continued treatment with 6-MP.

Bernard and Seligmann (28) report a series from Paris, France, in which 61 leukemias were treated with 6-MP. They were able to report a complete remission in 35 per cent of cases of acute leukemia of children not

previously treated. However, they had only an occasional remission in cases of acute leukemia of children grown resistant to antifolics and cortisone, and 6-MP therapy in acute leukemia of adults was not satisfactory. They did report satisfactory remissions in certain cases of chronic myelogenous leukemia.

Pierce (29), from the Bob Roberts Hospital for Children in Chicago, reports a series of 19 cases of acute leukemia of children in which the acute monoblastic cases responded better than the stem-cell type of leukemia. Bethell and Thompson (30), from the University of Michigan, reported that, in a series of 40 patients with leukemia treated with 6-MP, the better therapeutic responses were being obtained in young adults with acute hemocytoblastic and acute monocytic leukemia. Fountain (31), from Leeds University, England, presenting a series of 22 patients treated with 6-MP, demonstrated results comparable to those of Burchenal (25). Rosenthal et al.(32) report a series of 61 patients treated with 6-MP at Harlem and Mount Sinai Hospitals, New York. Hill and Lajous (33) report a series of 34 patients treated with 6-MP at Baylor University, Dallas. Again, these results are comparable to those of Burchenal (25).

Gaffney and Cooper (34) reported a series of 42 patients undergoing 6-MP therapy at the University of

Pittsburgh and the Children's Hospital of Pittsburgh. It was their opinion that therapy handled by a clinician must produce results rapidly, in contrast to the relative unlimited time allowed for results in investigative work. With this in mind, they felt that 6-MP alone was not the therapy of choice, as it requires over three weeks to show clinical response. Cortisone and/or antifolic drugs should be used as initial therapy for a rapid response.

Dosage and Toxicity of 6-Mercaptopurine

The recommended dosage of 6-MP has been established as 2.5 mgm. per kg. of body weight once daily. This is given orally in the morning. Burchenal (25) has given up to 12.5 mgm. per kg. of 6-MP daily for a period of a week with no apparent ill effects. However, it should be pointed out that most of the patients given these very large doses had been treated with ordinary doses for varying periods of time. This, therefore, raises the question as to whether a patient can develop a tolerance to the drug. Although there is no statistical proof, Burchenal feels that it might be possible to decrease the usual 3-to-8-week latent period in clinical response by giving a "high loading dose" of 5-10 mgm./kg. for one or two days.

According to Hall (27), the most common toxic

manifestations of 6-MP are anorexia (32 per cent), nausea (28 per cent), stomatitis (24 per cent), and vomiting (16 per cent). Diarrhea, proctitis, fever, rhinitis, swollen tongue, and melena have been reported to have incidence of about 4 per cent. Suppression of bone-marrow activity such as leukopenia, anemia, and thrombocytopenia is not considered to be a manifestation of toxicity, as it is an essential part of the therapeutic procedure. When evidences of ulcerative lesions of the mouth, or ulcerations of the lower gastrointestinal tract manifested by diarrhea or melena develop, administration of 6-MP should be discontinued. With healing of the lesions, therapy may be resumed in a lower dosage.

Most authors agree that, in the clinical management of 6-MP therapy, therapy should be discontinued with the onset of a leukopenia (25,26,27,29,30,31,32,33,34). However, Bernard (28) maintains that there is no necessity to discontinue therapy with marked leukopenia or necrotic lesions in the mouth. Two of his patients developed these complications, and therapy was continued with resultant healing of the stomatitis and a gradual return to normal of the leucocyte count. In one of his patients the count reached a low of 200 leukocytes/cu.mm. and returned to normal under therapy. Bernard's only explanation for his success is that he routinely gives

blood transfusions until the red-cell count reaches 4 million/cu. mm., before beginning therapy with 6-MP. It is of interest to note that we have no cross check on his work here, as all other authors have discontinued medication with the leukopenia.

Certainly, the toxic manifestations of 6-MP must be minimal, for deAsua (35) points out the much higher incidence of ulcerous stomatitis in the use of Aminopterin. This permits the prolongation of 6-MP therapy for any necessarily extended period.

There have been occasional reports of icterus with inconclusive liver function tests. The icterus cleared upon cessation of 6-MP therapy. Farber (26). Two instances of drug fever, with a syndrome of chills and fever up to 104° F., were reproduced on several occasions by the administration of a single dose of 6-MP in these hypersensitive patients. Several cases of eosinophilia with counts up to 54 per cent have been noted in patients under prolonged therapy. Hyman (36).

Criteria for Remissions

In order to evaluate properly the results of 6-MP therapy, certain criteria for remissions must be established. This is necessary in order that response to therapy is uniformly established by various authors.

The only difficulty in this respect is that, to date, no uniform criteria for remissions have been established. One of the more rigid classifications is that of Hall (27). He defines a complete remission as one in which there is a disappearance of the clinical manifestations of the disease, with a return of the blood and bone marrow to normal, with the bone marrow containing no more than 5 per cent of prolymphocytes, progranulocytes, or promonocytes. A partial remission is one in which there is an improvement in the clinical and hematologic picture, but evidence of leukemia yet remaining in the bone marrow. Bross (37), in analyzing the criteria for remissions used by twenty-one authors, offers a classification which is compatible with the thinking of most workers in this field. A "good hematologic and clinical remission" is a clinical remission with a return of the peripheral blood to normal, and a combined total of less than 30 per cent prolymphocytes, progranulocytes, or promonocytes in the bone marrow. A "partial remission" is clinical improvement with some improvement of periph-eral blood and bone marrow. A "failure" is little or no clinical improvement, with no evident response of periph-eral blood or bone marrow.

Clinical Management of Patients Under 6-Mercaptopurine Therapy

In patients 6-MP is given orally in the daily recommended dose of 2.5 mg./kg. body weight, as has been mentioned previously. Three to eight weeks of therapy are required to achieve remissions. The course of the patient during this time must be followed closely. Hall (27) recommends complete blood examinations three times a week, and bone marrow aspirations every week, or every other week. Tivey (3) believes that no single marrow aspiration site should be diagnostic of a remission as there are discrepancies in the number and type of leukemic cells in various sites tested. Therapy should be continued until leukemic leukocytes disappear from the bone marrow. This usually results in severe degrees of leukopenia with counts of less than 1000 leukocytes per cu. cm., but normal hematopoiesis is usually restored within two to three weeks after discontinuing the drug.

Burchenal (2) has pointed out the value of supportive therapy after discontinuing the drug. Anemia should be prevented with frequent transfusions. Antibiotics should be used to control infection. It is very important to maintain the morale of the patient. For this reason, the patient should be allowed to live as nearly a normal life as is possible within the limits

of his physical condition.

Most authors agree that it is not necessary to continue the use of 6-MP when a complete remission has been obtained. In these cases the patient is closely watched, and therapy resumed with first evidence of relapse. However, should the patient have a partial remission only, 6-MP may be given continuously or intermittently for long periods of time.

Combined Therapy

The comparison of the effectiveness of 6-MP with other antileukemic preparations is indeed difficult. There is no doubt but that it is effective, but is it more, or less, effective than other drugs such as the folic acid antagonists, cortisone, and ACTH? This question at the present time cannot be answered; there are too many controversial opinions presented. Most of the studies of 6-MP therapy have been on patients who have been on previous folic acid antagonist and/or cortisone therapy. In many cases 6-MP therapy was initiated as a last resort when the patient would no longer respond to the older medications.

However, there is no doubt about the effectiveness of combined 6-MP, folic acid antagonist, cortisone therapy. Burchenal (2) recommends the initial use of

cortisone or ACTH in acute leukemia if the patient appears unlikely to be able to survive the three weeks usually necessary for either 6-MP or the folic acid antagonists to take effect. However, in the less acutely ill patients, the antimetabolites may be used initially. Burchenal (2) believes that Amethopterin is the drug of choice if the patient is a child under ten years of age with a white count below 50,000. With Amethopterin there may be a somewhat longer remission. However, 6-MP may offer a better chance for remission if the white count is above 50,000, or if the patient is an adult. It is best to continue the use of a particular antimetabolite as long as a remission can be maintained. At the first sign of failure to respond to the drug, therapy should be switched to the other antimetabolite. Exacerbations of the leukemia, which are not controlled by the antimetabolites, should be controlled if possible with ACTH or cortisone.

De Asua (35) maintains that only one antimetabo-lite should be used at a time in order to avoid the pos-sibility of simultaneous resistances to the drugs. Farber(26) has seen no evidence of earlier onset or of longer remissions in cases where both types of antimetabolites were used simultaneously. Other authors report the value of alternate 6-MP and Amethopterin or cortisone therapy.

The reader is encouraged to consult the literature forthese many reports.

Increase in Survival Time

As was first mentioned in this paper, leukemia is a fatal disease. However, it has been pointed out that there are now specific drugs which alter the normal metabolism and progressive course of this malignancy. Although there is no known cure at the present time, there has been a marked increase in survival time since the turn of the century. X-ray, cortisone, ACTH, and nitrogen mustard have been used in the various leukemias. Vigorous supportive therapy has increased chances of survival. But the antimetabolites developed since 1948 have been the first real hope in the treatment of this disease. Rice (38) has reported a series of 103 leukemic children. These patients were seen over a 16-year period at Children's Hospital, Washington, D.C. Before the advent of Chemotherapy (1938-1947), the average survival time following the diagnosis of leukemia was 16 weeks. The average survival time at present is 49 weeks. Other authors, like Rice, have reported significant increases in survival time with antimetabolite therapy. Farber (26), considering 6-MP therapy, reports an average duration of improvement of 8 weeks following the failure of

continued remissions under the therapy of the folic acid antagonists.

Burchenal (2) makes the following plea for the vigorous treatment of all acute leukemias:

> Too often the attitude of the doctor is that there is no cure for the disease, therefore the child or adult should be allowed to die in peace. The answer to that is fourfold:
> 1) The untreated patient does not usually die in peace but frequently suffers many of the painful complications of the disease;
> 2) under therapy many of these children can live happy lives, continuing in school and playing as normal children;
> 3) during this time the family have time to adjust to the situation; and
> 4) most important, if these patients can be kept alive for another year or two, who can say that some investigator somewhere in the world will not come forth with a really effective agent to control the disease?

VI

SUMMARY

A description of the clinical and pathological course of leukemia has been presented, along with a brief discussion of some of the various methods of treatment. The relationship of adrenal stress to spontaneous remissions of leukemia has been considered, as well as the theory on the mechanism of action of anti-metabolites.

In order to understand the mechanism of action of 6-Mercaptopurine in the therapy of leukemias, a discussion on the synthesis of the nucleic acids has been presented. Adenine and guanine are essential substances in the synthesis of nucleic acids. 6-Mercaptopurine has a blocking effect upon hypoxanthine, having a chemical structure very similar to that of the purines. In this manner, it inhibits the normal production of adenine and guanine.

The chemical synthesis of 6-Mercaptopurine has also been presented. Experimental data regarding the effect of 6-MP on various experimental animals and experimental tumors have been presented, along with studies of the drug's toxic effects and metabolic pathways.

Finally, a brief review of some of the available clinical data on 6-MP has been presented. An effort has

been made to correlate the results of 6-MP therapy by the various authors with respect to indications for use, dosage and toxicity, criteria for remissions, clinical management of patients, combined therapy, and increase in survival time.

6-Mercaptopurine is indicated for therapy of acute leukemias and chronic granulocytic leukemia. However, Myleran may prove to be of more value in chronic granulocytic leukemia, and the folic acid antagonists are of equal value in acute lymphocytic leukemias. 6-MP is administered in daily dosages of 2.5 mg./kg. orally, adjusting the dosage according to response. Response is followed by leucocyte counts and bone marrow studies. Toxic effects other than those of hematologic depression are related to ulcerations of the gastrointestinal tract. Remissions are classified as complete, partial, or failure, depending upon the clinical picture, and blood and bone marrow studies. Medication is continued during partial remissions, and discontinued during complete remissions. If resistance develops to 6-MP therapy, the folic acid antagonists or cortisone should be substituted. Under this program, a patient with acute leukemia may be expected to survive with reasonable comfort for almost a year after diagnosis of the disease.

CONCLUSION

6-Mercaptopurine has been shown to be a valuable addition to the antimetabolite therapy of leukemias. This may be one of a series of drugs leading to the eventual discovery for a cure for leukemia.

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