5-1-1940

Hemolytic anemia and agranulocytosis associated with the administration of the papa-amino-benzene sulfonamide group of drugs

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HEMOLYTIC ANEMIA AND AGRANULOCYTOSIS ASSOCIATED WITH THE ADMINISTRATION OF THE PARA-AMINO-BENZENE SULFONAMIDE GROUP OF DRUGS

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Senior Thesis

Presented to

The College of Medicine
University of Nebraska
Omaha

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TABLE OF CONTENTS

INTRODUCTION

Five years have elapsed since Domagk (19) announced that he had found that prontosil had an elective action against experimental streptococcal infections in mice and rabbits. At the conclusion of this interesting report he says, "Prontosil zeigt eine bisher am infizierte Tier nich beabachtete Streptokokkenwirkung. Es gelingt, streptokokken infizierte Mäuse, die unbehandelt innerhalb von 24 Stunden nach der Inferktion eingelen, durch subcutane und perorale Prontosilsgaben am Leben zu erhalten. Prontosil zeight eine elektive Wirkung bei der Streptokokkensepsis der Mäuse". In carrying out his experiments he observed that prontosil was not effective in vitro in preventing the growth or killing cultures of streptococci. He did not consider the drug toxic for he observed no signs of toxicity while he was testing the efficacy of the drug in combatting staphylococcal and streptococcal infections in laboratory animals.

His work was confirmed during the same year in France and later in 1935 Trefouel, Trefouel, Nitti, and Bovet (100) found that para-amino-benzene sulfonamide, the nucleus of the prontosils, was the active principle by demonstrating that it is effective in controlling

and curing experimental streptococcal infections in animals.

Interest in these new chemicals became actively stimulated on the continent and new chemicals with the para-amino-benzene sulfonamide nucleus were synthesized. More experimental work on experimentally infected animals was carried on, and the clinical use of the drug increased.

The medical professions both in America and England were apparently unaware of the fact that an important group of drugs had been found to have extraordinary power in curing both experimental and clinical streptococcal infections. This is explained by the fact that people naturally pay more attention to the journals written in their own tongue. In addition to this, the drugs were difficult to obtain in large enough quantities to carry on any significant work because American manufacturers had not yet perfected suitable methods of synthesis to make available cheap supplies of these drugs to investigators.

This unawareness, however, came to an end after Colebrook and Kenny (13) and Buttle, et al, (11) of England, and Long and Bliss (60) made their reports concerning the effects of this group of drugs in 1936. Since that time, the para-amino-benzene sulfonamide

(sulfanilamide) group of drugs has been very widely used for treating both clinical and experimental infections. Clinically and experimentally they have been employed for practically every known infection. It is no wonder that the statement is so often made that patients are first treated with sulfanilamide and if this has no effect upon the course of the illness a history of the illness is obtained and a physical examination of the patient is made to discover the nature of the patient's complaint. With such widespread use, both the beneficial and deleterious effects of these substances could be observed repeatedly.

These drugs have, without doubt, brought about a wonderful advance in the treatment of bacterial infections. In the case of such substances, it is relatively easy to become too enthusiastic over their effect and ascribe to them the properties of a cure-all. That these substances have been found to have toxic effects was not unanticipated since their nucleus is the benzene ring with an amino-group attached. Such drugs have long been known to be responsible for toxic effects upon the blood picture and hematopoietic tissue.

In comparing the incidence of serious toxic effects of the drug upon patients to those cases in which no serious toxic changes occur, it is apparent

that the percentage is small. Due to the fact that the drugs do have toxic effects should discourage their promiscuous use in conditions where there is no real indication for their use. Their use, however, should not be neglected in those cases in which it has been proved that they are curative and beneficial, e.g., streptococcal, meningococcal, gonococcal, and pneumococcal infections.

One of the most interesting toxic effects is the change which occurs in the blood picture of both animals and patients given these drugs. Our interest has been stimulated to some extent by the results obtained in a few animal experiments at the University of Nebraska (64) and also by the interesting reports of blood dyscrasias which have been increaseing ever since the reports by Young (108) and Jennings and Southwell-Sander (46) have been published. In this thesis a brief review is being made of agranulocytosis and hemolytic anemia associated with the administration of the drugs of the para-amino-benzene sulfonamide group.

THE RESULTS OF EXPERIMENTAL WORK ON ANIMALS

When Plumer (77) reported in April, 1937, a death due to leucopenia following treatment of subacute bacterial endocarditis by sulfanilamide, the experimental production of blood changes produced by giving the drug to experimental animals had not yet been accomplished. Hawking (36) was unable to produce blood changes. Marshall, et al, (69) published their work concerning the toxicity of sulfanilamide in January 1938. They had fed 0.2 grams of sulfanilamide per kilogram to two dogs for two months and had not found any change in either the red cell, white blood cell, or in differential blood cell counts. Large doses of sulfanilamide were given to two dogs and four rabbits to determine whether sulfanilamide affected the blood count when given in large doses in a few days time. These doses ranged from 3.5 to 6 grams of the drug per kilogram in 2 to 5 days time. No effect upon the blood picture was observed. Hageman (33) gave 20 grams of sulfanilamide to a group of mice in 14 days time. Since he found that the spleens of these mice had much hemosiderin deposit and that the eosinophils of the bone marrow had increased, he suggested that the drug was able to destroy red blood cells and cause an allergic response

of the bone marrow. Finkelstone and Sayliss (26) noticed abnormal erythrocytes and nucleated red blood cells in the blood of rabbits to which he fed 1.2 grams each day.

Domagk (19) was unable to produce any changes in the blood of experimental animals with prontosil and neo-prontosil. In 1937 he (20) found that sulfanilyl sulfanilamide and various derivations of that drug were able to cause various changes both in the white and blood cell picture. These changes were inconsistent as far as the white cells were concerned. The mono-methyl derivative of sulfanilyl sulfanilamide caused the red blood cell count to drop when given to laboratory animals. Bone marrow studies were not made to determine the reaction of the bone marrow to the drug.

Kreutzman and Carr (57) gave 8 rabbits doses of prontosil for a period of 21 days. The dosage was slightly higher than the recommended dose. The peripheral blood counts did not consistently change throughout the experiment but the red blood cell counts increased from 10 to 15% after the drug had been discontinued. Spleens were moderately congested and the single lobe eosinophils of the bone marrow were increased. These findings were interpreted to be suggestive of a mild bone marrow depression caused by the continued

administration of prontosil.

When Whitby (103) introduced sulfapyridine he thought that it would produce fewer toxic effects than sulfanilamide. Wien (104) gave 0.25 mgs. per gram of sulfapyridine to four rats for 14 days and 0.5 mgs. per gram to another group of four rats for 2 weeks. He used the same size dose of sulfanilamide for two other groups of rabbits for the same length of time. Two blood counts were made each week and no changes were found in the peripheral blood picture. No changes in the blood pictures were found in cats given 1 gram of sulfapyridine in 7 days or in dogs given an identical dose. When he fed larger doses of sulfanilamide and sulfapyridine, 1.5 to 3 mgs. per gram, to rats for 15 days, he found that those animals receiving sulfanilamide became anemic, had an increased amount of urinary porphyrin output, but those that had received sulfapyridine did not develop such changes.

During 1938 some of the effects of both small doses of sulfanilamide given over a period of five weeks and large doses given to rabbits were observed at the Nebraska University College of Medicine (64) 15 rabbits were given 4 grams of sulfanilamide per kilogram in a 24 hour period. At the end of the sixth day after the initial dose 13 rabbits were dead. The two

remaining rabbits were killed. The red and white blood cell counts showed no consistent change. The differential counts shifted to the left and the reticulocyte counts increased. Counts and studies made of the bone marrow stained by the panoptic stain revealed no significant alterations. Bone marrow counts of 8 normal rabbits were made in order to have a basis of comparison for the bone marrow counts made on the marrow from the experimental animals. In studying the chronic effects of sulfanilamide we made observations on two groups of rabbits. One group of four received daily doses of sulfanilamide, 1.0 gram per 9 kilograms of weight, for a period of 35 days. The other group, consisting of five rabbits, received the same dose, but each rabbit had a five-hour fever treatment each week throughout the course of the experiment. Studies of the peripheral blood throughout the experiment were made at weekly intervals and it was found that the small daily doses of the drug had no consistent effect upon the blood picture. Of the group of four, one animal died of pneumonia. The same dosage of sulfanilamide combined with fever did, however, result in blood changes in 3 rabbits. One rabbit was killed accidentally during the second week but no blood changes had developed at that time. Of this group the one rabbit

that was living at the close of five weeks had had no blood cell changes. The other 3 rabbits died 10, 15, and 24 days after the beginning of the experiment. Two of these rabbits had a leucopenia at the time of death with decrease in all the cellular elements. The other rabbit also had a leucopenia but the great reduction in the total count was due to the fact that only 2% of the cells were granulocytes. Unfortunately our bone marrow preparations were unsatisfactory.

During 1939, several interesting reports concerning the effects of drugs of the para-amino-benzene sulfonamide group were made. Rosenthal (86) and Nelson (73) studied the effects and histopathological changes in hens and rabbits following administration of sulfanilamide and sulfanylil sulfanilamide to rabbits and chickens. They found that these drugs both have cumulative toxic effects for both rabbits and hens. Bone marrow from 11 rabbits that had received repeated doses of sulfanilamide were examined. Of 11 preparations studied, 3 were normal, 3 slightly hypoplastic and 5 were moderately hyperplastic. In 3 of the hyperplastic bone marrows the proportion of early forms of cells to late forms was normal. One hyperplastic tissue was found to contain increased numbers of stem cells and early granulocytic forms and the fifth of this group of hyperplastic

marrows showed that an increase in the mature forms of granulocytes had occurred. It was found that one of the hypoplastic tissues had a reduction in the number of normoblasts. The bone marrow of rabbits treated with disulfanilamide had less pronounced changes occur after the drug had been administered in repeated doses. Of 9 bone marrows studied, 6 were normal. Of the three which were abnormal, one had a hypoplasia of granulocytes, one a hypoplasia of normoblasts, and one bone marrow preparation had a hyperplasia of immature granulocytes. All the bone marrow studies made on the hen bone marrow given either sulfanilamide or disulfanilamide in repeated doses showed hypoplasia.

Later in 1939 Rosenthal and Bauer (87) were able to demonstrate hydroxylamine derivatives in the urine of rats, rabbits, dogs, and man after they had had sulfanilamide administered to them. This was found in larger quantities in the urine of man and rabbit than in the other animals. These oxidation products were found to be of greater toxicity than the original drug administrated.

Molitor and Robinson (72) in 1939 published the results of their study of acute, cumulative and chronic toxicity as well as pharmacological effects of

sulfanilamide and benzyl sulfanilamide upon 1980 animals. Dogs to which they gave sulfanilamide for 3 week periods had no blood picture changes. In every case they observed that sulfanilamide was very much more toxic than benzyl sulfanilamide. They had no deaths of animals fed as much as 45 grams per kilogram for 10 days. Pathological changes they found in the tissues of animals which are significant were large, hyperemic, pigmented spleens. The pigment was both iron and non-iron containing substances. The bone marrow was moderately hyperplastic. Pigment was also present in the lymph glands and in the convoluted tubules of the kidneys. These findings indicated that a destruction of blood elements occurred which was responsible for increased stimulation of bone marrow.

That sulfapyridine is harmless and non-toxic for mice was concluded by Johannsen (47) during his investigation of the toxic and therapeutic effects of the drug. This is not the same conclusion which Molitor and Robinson as well as Antopol and Robinson (3) reached during their experiments with sulfapyridine with various kinds of laboratory animals. Bone marrow hyperplasia of the myelogenous elements as well as lymphoid hyperplasia and pigmentation of the spleen was found. Peripheral blood studies were not made in these

experiments.

Very interesting is the work reported by Higgins and Machella.(37) They induced anemia in rats in a period of 10 days to two weeks by using doses of 1 gram of sulfanilamide to 1 kilogram of weight. They attributed the anemia due to a direct effect the sulfanilamide has upon the blood cells of the rat. They found coproporphyrin in the urine of rats and say that its presence indicates a bone marrow derangement. When they gave rats the same dose of sulfanilamide in connection with five 50 mg. doses of nicotinic acid, anemia developed as it had when the drug was administered without the nicotinic acid. Bone marrow studies were made. No impaired hemopoiesis was found. There was some erythroid shift but the essential structure of the bone marrow was not impaired.

Machella and Higgins (65) have been able to induce anemia in rats by administering doses of varying quantity to different groups. Their work is especially interesting since very similar cases have been reported as a result of the therapeutic use of the drug in treating infections in man. The experiment and its results are described briefly. They used four different doses of sulfanilamide on each of four different groups of

rabbits. For convenience, a list of the dosage and groups is included.

Dosage	No. of Rats in Group
0.25 gms. per Kg.	6 rats
0.5 gms. per Kg.	6 rats
1.0 gms. per Kg.	12 rats
2.0 gms. per Kg.	12 rats

They were able to induce anemia in these rats in which the degree and rapidity of onset were dependent upon the size of the dose given. A list of the results they obtained is included.

1. Increased color index.

2. Anisocytosis and macrocytosis.

3. Rapid decrease in red blood cell count and hemoglobin. Degree and rapidity of drop dependent on the size of the dose.

4. Rats were thirsty, cyanotic, drowsy and irritable during the onset and throughout the course of the drug administration after changes had begun.

5. Initial drop in white blood cells was followed by leukocytosis.

6. Steady and progressive drop in the total hemoglobin also was observed.

7. In the bone marrow, the erythroid and myeloid cells were increased. A transient increase in eosinophilic metamyelocytes was also noted.

8. Examination of tissues was made . Findings were hemorrhagic gastritis, black spleens enlarged lymph glands, and dark livers. These animals had a gradual recovery from the anemia after the drug was discontinued.

In summarizing the work of the effects of various sulfonamide drugs upon the blood cells and hemopoietic systems it is concluded that these drugs do affect the blood cells and the hemapoietic tissues. The presence of increased amounts of pigment in the spleen and in some cases in other tissues of the body indicates that an increased amount of red blood cell destruction occurs when these drugs are administered. That the red cell count and hemoglobin does actually decrease was indicated by Machella's and Higgins' (37)(65) work. There is little work showing changes in the peripheral white blood cell counts since most of the work of investigation of the harmful effects of these drugs on experimental animals did not include blood counts. Since the bone marrow did have an altered picture in most instances, it is reasonable to conclude that had the peripheral blood picture been studied, the

changes found during histopathological studies of the bone marrow would have been reflected by the peripheral blood.

AGRANULOCYTOSIS AND HEMOLYTIC ANEMIA: SENSITIVITY REACTIONS OF THE BONE MARROW.

Introduction

Since 1937 the number of reported cases of hemolytic anemia and agranulocytosis associated with or following therapy with sulfanilamide and allied compounds has increased. That this should occur is natural since the total number of patients treated constantly increases and provides opportunity for more blood disturbances to develop. Undoubtedly the few observations of blood changes associated with these drugs in animal work is due to the fact that the course of the effect of the drug has not been so closely watched as in human beings treated with the substances, nor have as many animals been exposed to the drugs as human beings.

Hemolytic Anemia

Before considering the red blood cell and white blood cell disturbances following the use of the sulfonamide drugs, a brief review of the nature or character of the conditions known as hemolytic anemia and agranulocytosis is given.

Hemolytic anemia is a condition characterized by hyperbilirubinemia, urobilinuria, hemosiderosis,

reticulocytosis and normoblastic hyperplasia of the bone marrow, and a high grade of leukocytosis if large numbers of red blood cells are suddenly destroyed.

It may be due to peripheral hemolysis caused by a direct hemolytic effect of certain chemicals, bacterial toxins, and drugs or due to an idiosyncratic effect caused by complex antigen-antebody reactions of foreign proteins or various chemical compounds.(27) Examples of direct hemolytic poisons are phenylhydrazine trinitrotoluene, hydrogen arsenide, n-propyl disulfide, snake venoms, saponins, and bacterial toxins. Fitzhugh (27) says that the hemolytic effect of dinitrophenol, acetanelid, and antipyrine is due to an individual idiosyncrasy of an occasional person, for these substances do not cause a hemolytic anemia in the larger percentage of people to whom the drug has been given. Since the advent of sulfanilamide, both Long (60)(61)(62)(63) and Fitzhugh (27) think that the hemolytic anemia which develops during sulfanilamide therapy is a manifestation of an anaphylactic or idiosyncratic effect which the drug has on an occasional patient.

It is admitted that hemolytic anemia is due to complex sensitivity reactions but the factors which are concerned in the actual production of an occasional

sensitivity are not known. This is true not only for this type of allergy but for all allergic phenomena. Factors to be considered are heredity, hormonal mechanisms, dysfunction of liver and spleen, fatigue states, traumatic shock, toxemias which include putrefactive disturbances and various bacterial and protozoan infections, deficiency states. Radiation effects, also are to be considered. Fitzhugh (27), Jackson and Parker (43), Kracke and Parker (55), Madison and Squier (66), Pepper (76), and Rosenthal (84) all feel that these factors are to be considered as etiological agents in the production of various sensitivity reactions of the blood and bone marrow.

Agranulocytosis

Introduction. In 1922 Schultz (90) reported a condition which he named agranulocytosis. The disease he described was characterized by an acute onset, with chills, fever, jaundice, ulcerative and gangrenous lesions in the mouth, and leucopenia with granulocytopenia. All of the six cases which he reported occurred in middle-aged women who had been in good health. The six cases were all fatal. According to most workers who have investigated and observed agranulocytosis since that time, this is regarded as a new disease. (27)(22)(23)(45)(55)(66) Pepper(76), however, thinks that the

condition, formerly known as putrid sore throat, was an analagous condition. The other workers feel that since blood counts have been done for over 50 years, certainly the disease would have been reported before 1922 had it actually existed.

Definition. Before the discussion of agranulocytosis or agranulocytic angina is continued, the condition should be defined. At first in glancing through the literature one is confused by the large number of different terms used to name the symptom complex. The expressions acute granulocytic angina, agranulocytosis, malignant granulopenia, and acute primary granulocytopenia are synonomous terms according to Jackson and Tighe.(44) Kracke (53) says that the term agranulocytosis, strictly speaking, is a misnomer for it means an increased number of immature granular cells. Fitzhugh (27) suggests the term pernicious granulocytopenia. For the purpose of avoiding confusion in this discussion, the term agranulocytosis is being used, and the condition is defined as an acute disease characterized by extreme leukopenia and granulopenia and systemic reactions which in turn may be followed either by no infection, localized infection, or generalized infection.

Etiology. The exact etiology is unknown. There are, however, predisposing etiological factors. Pepper (76) has observed that there is a higher incidence of this disease among allergic patients than in non-allergic people. Kracke (54) is well known for his work both in the clinical investigation of the disease as well as the experimental production of the condition by various substances such as benzene, hydroquinone, and ortho-oxybenzoic acid. He says it is a disease of the myeloblastic tissues followed by a loss of resistance, resulting in overwhelming infection, but the exact etiological agent is not known. Madison and Squier (66) were able to cause a fatal agranulocytosis in 1 of a group of 11 rabbits, by benzene drugs and feel that these drugs are in some manner responsible for a sensitization of the bone marrow which results in sudden leucocyte decrease. That drugs are responsible for the condition, especially those of the benzamine group, is the most tempting of theories, but at times cases of agranulocytosis develop when there is no history of the use of drugs of this group. Kracke (55) quotes the work of Plum who made a very extensive survey of the incidence of agranulocytosis in Denmark. He was able to correlate the incidence of agranulocytosis with the incidence of use of aminopyrine. The greatest

incidence of the disease occurred during 1933 and 1934 when the largest amount of the drug was being consumed in Denmark. This was well demonstrated by a graph he constructed in which it was demonstrated that the curve representing the use of aminopyrine and that representing the incidence of agranulocytosis were parallel.

Another interesting fact regarding the incidence and distribution of agranulocytosis is that 90% of the cases occur in the United States and Germany. (44)(53)(55)(76) This corresponds to the area in which aminopyrine was most frequently used a few years ago. By the time sulfanilamide became a populor therapeutic drug, the incidence of agranulocytosis had decreased greatly associated with the lessened use of aminopyrine. It is as yet too early to measure the effects that the introduction of this new product will have upon the total incidence but, judging by the increasing number of reports of agranulocytosis associated with therapy with this group of drugs, it is very likely that the incidence has made a very definite increase.

The hormonal factor as an etiological factor in the production of agranulocytosis is suspected to play a role because the condition occurs most often between the ages of 30 and 40. The ratio of the

incidence occurring in women and men is 3 to 1, the greatest percentage being found in women.

That the standard of living has an effect is well borne out by the fact that before sulfanilamide was introduced, the occurrence of agranulocytosis was most common in that class of people of higher economic standards.(55) Of this group nurses, physicians, and members of the family of physicians were found to have the disease most frequently.

When Schultz (90) described agranulocytosis he said that the cause was due to an acquired sensitivity of the leukocytes, endothelium, and leukopoietic tissues to certain drugs or other allergens. Despite much observation and investigation since that time, little more can be said in regard to the disease today. If it is a bone marrow idiosyncrasy does it result in a lack of a maturation factor or a lack of a chemotactic factor which interferes with the delivery of the blood cells into the vascular system? Fitzhugh (27) calls the condition pernicious granulocytopenia for he believes that the condition is caused by an arrest in the maturation of granulocytes which is analagous to the arrest of maturation of erythrocytes in pernicious anemia. He believes this to be true because he has been unable to find a decrease in the myeloid cells of the

bone marrow, but has found a hyperplasia of the early forms of granulocytes. Furthermore, the lesion of the leukopoietic tissues is reversible to a certain point if it has not continued so long that aplasia finally results. Remissions of the disease are characterized by an outpouring of young cells before the mature forms appear. Fitzhugh has said that possibly the sudden onset is due to the fact that the inciting factor may cause the cells to stick to the endothelial lining and thereby cause a sudden marked drop in the cell count. That no evidence of abnormal distribution of granulocytes is found during histopathological studies is explained by the fact that the adherent cells disintegrate rapidly.

Overwhelming septic conditions do occasionally result in a profound as well as fatal leukopenia associated with a profound reduction of the granulocytic cells. It is the opinion of Kracke (53)(54), Pepper (76) Jackson (43)(44), Madison and Squier (66), and Fitzhugh (27) that the sepsis which is often associated with agranulocytosis is not the actual cause but rather the result of agranulocytosis which depletes the resistence of the tissues to infection by the lack of defensive agents. Domagk (21), however, does not believe that true agranulocytosis develops as a result of the

administration of the amino-benzene sulfonamide compounds but that the granulocytopenia is the result of the effect of the etiological agent producing the disease for which the drug is being used. That a granulocytopenia can develop in response to a severe infection is true. Schilling (89) says that this is either brought about by the overwhelming irritation of a severe acute sepsis or persistent drain of a long continued infection. In the case of sulfanilamide, however, it is hardly possible to believe that the infections have been so much more severe during the past few years that the infection itself and not the sulfonamide drugs have been responsible for the increased number of reports of agranulocytosis. In this connection the case reported by Ives (42) is very interesting. A negro patient after nine days of illness with hemolytic streptococcic angina had a total white blood cell count of 9000 with a complete absence of granulocytes. The patient had a grayish black necrotic membrane over his pharynx, he was confused, drowsy, and complained of a headache and joint pain. The lowest white count was 1200, with no granulocytes one day after hospital admission. 5 cc. of prontosil every four hours and 60 grains of sulfanilamide as well as intramuscular liver extract and intravenous pentnucleotide were

administered as soon as the blood findings were made. Recovery began on the second day after beginning of therapy and the patient had an uneventful course of illness in the hospital. In this case the sepsis was overwhelming enough to cause a severe granulocytopenia, and the drug was indicated because the toxic, septic process had to be controlled.

Pathology. The pathological changes of agranulocytosis are located chiefly in the bone marrow. If angina occurs, necrosis and ulceration without evidence of granulocytic invasion occur in the mucous membranes, chiefly those of the tongue, nasopharynx, and pharynx. Necrotic lesions are occasionally found in the vagina, gastro-intestinal tract, and bladder.

The bone marrow changes are the most important pathological findings of this disease. Kracke (53), Jackson (44)(43), Fitzhugh and Krumbhaar (28), and Darling, et al., (22) find that there is a maturation arrest with hyperplasia at the stem cell stage of the myeloid elements of the bone marrow if the disease is rapidly fatal. If the disease is prolonged there is an increased number of plasma cells and lymphocytes with hypoplasia of the myeloid cells. There is, however, no evidence of degenerative changes occurring in the bone marrow cells. If the bone marrow

is degenerated it is either the result of an infection or poor fixation of the tissue. Bone marrow from patients who are recovering from the infection has increased numbers of more mature cells. Rosenthal (85) finds that the bone marrow is aplastic. Pepper (76) also believes that an aplastic as well as a degenerated bone marrow is found in these conditions. It is possible, as Jackson (44) says, to misinterpret bone marrow findings, especially if the tissues have been improperly fixed. It is also possible that Pepper (76) and Rosenthal (85) both examined bone marrow from patients with a prolonged course of the disease. Jackson (44) also thinks that the original report of the bone marrow findings by Schultz (90) are also frequently misinterpreted to mean aplasia of the bone marrow instead of a maturation arrest. To us it seems that the conclusion may be reached after having examined the reports of the various authorities that the bone marrow may be either aplastic, hyperplastic, or hypoplastic.

Course of Disease. Clinically, the course of the disease has an acute onset. Often there is a prodomal period of malaise. The fever is high, ranging from 104° to 107° F. The patient is prostrated, usually complains of severe headache, has chills and a sore throat. Often there are ulcerative lesions both in the nose, mouth

and throat which have a peculiar gray green appearance with very little or no inflammatory change. The breath is foul. Along with these findings and symptoms there is extreme leukopenia with a great reduction in granulocytes. Often no granulocytes are found in the peripheral blood and the total count may be as low as only a few hundred cells. A few reports told of counts less than 100 white blood cells per cubic millimeter. Often positive cultures are obtained from the blood, but the bacteria are not regarded as the etiological agents of agranulocytosis, but the result of invasion of the body tissues as a result of the absence of the defense barrier which the granulocytes provide in protecting the body against bacterial infection. The disease has a rapid course and may terminate fatally in from 2 to 6 days or recovery when it occurs is complete in 2 weeks. Jackson (44) reports a 78% mortality. Kracke (53) found that 85% of 250 patients affliced with agranulocytosis died. Madison and Squier (66) observed 14 cases following aminopyrine injection. Of this group 8 died. It is uncertain what the exact mortality rate is, but there is no doubt that it is high.

Diagnosis. Diagnosis is based upon the following findings: Hemorrhage does not occur, few

immature white blood cells are present in the blood at the height of the disease, neither the liver nor the spleen are enlarged, and lymphadenopathy does not occur except that explained by local areas of sepsis. In addition to these findings recovery, if it occurs, is complete in two weeks, relapses are uncommon. Usually careful questioning and investigation will elicit the fact that some form of a benzene or aminobenzene drug has been used. Bone marrow changes may be an aid to diagnosis, but if we go on the assumption that it may be aplastic, hyperplastic or hypoplastic, it is necessary to correlate all the findings to arrive at a definite diagnosis.

Differential diagnosis may be very difficult. (44)(45) Other conditions from which it must be differentiated are overwhelming infections; benzol, gold, bismuth and arsenic poisoning; acute leukemia; aplastic anemia; and acute infections such as typhus, measles, mumps, influenza, malaria, dengue fever and roseola which characteristically have low total white counts. Careful blood studies, thorough examination, and complete histories are all valuable aids in establishing a diagnosis. An aplastic bone marrow with blood findings indicating a severe anemia, thrombopenia, and neutropenia is typical of aplastic anemia. If there is

a history of administration of gold, arsenic, bismuth, or benzol before the onset of such findings the etiology of the aplastic anemia is established.

Acute infections (53) seldom cause much diagnostic difficulty because of characteristic signs and symptoms which do not harmonize with the blood dyscrasias. Both acute leukemia as well as a neutropenia due to an overwhelming sepsis may be very difficult to differentiate from agranulocytosis. The neutropenia associated with sepsis is termed agranulosepsis by Kracke.(53) This condition may follow surgical procedures (81)(102) as well as acute infections (42)(9) and it may be very difficult to determine whether the symptoms and findings are pathognomonic of an idiopathic agranulocytosis or of an exhaustion or intoxication caused by the septic process. A bone marrow study may give the only clue by degenerative and toxic changes found within the cells. Severe septic processes of this type are apt to have an associated secondary anemia which may also be helpful in establishing the true diagnosis.

Jackson (45) warns of the ease in which acute leukemia and agranulocytosis may be confused. Only careful study of the patient's hematological picture correlated with the physical findings will, in some cases result in a definite diagnosis of either one of these

HEMOLYTIC ANEMIA ASSOCIATED WITH SULFONILAMIDE AND RELATED COMPOUNDS

Introduction

Both mild, slowly progressive hemolytic anemia and acute hemolytic anemia with a sudden drop in hemoglobin and red cells are associated with the administration of sulfanilamide and sulfapyridine.

Progressive Hemolytic Anemia

Long (63)(59)(61) and Wood (106) as well as Garvin (30) have all observed that a progressive hemolytic anemia with a fall of from 10% to 20% hemoglobin occurs in some patients. Jennings and Southwell-Sanders (46) made the first report of a slowly progressing hemolytic anemia associated with sulfanilamide administration. This is most often noticed in those patients who have received the drug for several weeks and most often appears after the tenth day. The reticulocyte count increases and there may or may not be a slight urobilinuria. All of these three workers make their statements after having observed many patients, but they do not state the percentage of patients treated over a long period of time that developed the low grade, chronic hemolytic anemia

Incidence. Bigler, Clifton, and Werner (7)

studied the blood picture of 33 patients while receiving sulfanilamide. They observed no severe anemia, nor did they find that any of their patients developed a low grade anemia. In fact, they say that often the hemoglobin of their patients apparently increased. Counts were done two times each week during a period of 28 days on 50 ambulatory patients being treated by sulfanilamide by Britton and Cowkins.(10) The dosage prescribed was 0.5 gram three times a day for 14 days. During that time they detected no evidence of a hemoglobin or red cell decrease. Hageman (32)(34) in reviewing 114 cases treated with sulfanilamide found one case of hemolytic anemia. Campbell (12) observed the blood picture of 10 cases being treated with sulfanilamide. Anemia was not found but the reticulocyte count increased. It is possible that destruction of the red blood cells did occur, but it was so slight that anemia was not detectable because the bone marrow was able to replace red cells as fast as they were destroyed.

Long, Bliss, and Feinstone (63) have observed that chronic anemia may also result when sulfapyridine is used. Hodes, et al., (39) studied 71 cases of pneumococcic pneumonia treated with sulfapyridine and found no significant hemoglobin change. Barnett, et al., (5) report no anemia from sulfapyridine therapy in a

group of 23 cases. No anemia is reported by Evans, et al., (25) who used sulfapyridine to treat pneumonia. Rosenthal and Vogel (84) who use the same drug have found that hemolytic anemia does occur.

Discussion. This type of anemia is not regarded as a grave complication of sulfanilamide therapy. Long and Bliss (59)(61)(62)(63) do not regard this complication of severe enough consequence to discontinue the drug unless the hemoglobin has dropped below 60%. The blood regenerates quickly if the drug is stopped and if the infection is not yet controlled and the hemoglobin has dropped to the 60% level they advise continuation of the drug with a blood transfusion.

Acute Hemolytic Anemia

The acute hemolytic anemia which develops is both spectacular and serious. Harvey and Janeway (38) made the first report of this toxic effect of sulfanilamide. The early symptoms, before the anemia develops, are nausea; dizziness; increased urobilin; fever; and symptoms of toxicity.(106)(30)(61) Other findings reported by these workers, as well as those whose cases are tabulated in Table I, are a rapid, and pronounced fall in the hemoglobin and the number of red blood cells. The count may drop as much as

TABLE I

Tabulation of Cases of Hemol
Drugs of the Para-Amino-Benze

Reference	Sex Age	Total Drug	Total Days	Diagn
Antopal Applebaum Goldman	Male 34	8 gms. Prontosil	3	Pneur
Antopal Applebaum Goldman	Male 5	90 gms. Sulfan.	36 Hours	Tonsi
Kaletsky	Female 64	24 gms. Sulfan.	4	Mastc Mastoi
Rosenthal Vogel	Male 9	9 gms. Sulfapy.	3	-
Wood *	Male 28	44.66 gms. Sulfan.	6	Stre Pneur

* Bone marrow was very hyperplastic w
proliferation.

TABLE I (Con

Tabulation of Cases of Hemol;
Drugs of the Para-Amino-Benze

Reference	Sex Age	Total Drug	Total Days	Diagn
Wood(106)	Male 36	21 gms. Sulfan.	5	Tonsi
	Same Pt. 1 Yr. later	7.8 gms. Sulfan.	3	Tonsi
Rosenblum Rosenblum	Male	2 gms. Sulfan.	2	Tonsi Ot. M
Nelson Scott-Young	Female 32	48 gms. Sulfan.	23 Days	Gonor
Harvey Janeway	Male 36	22.8 gms. Sulfan.	5	Strep Sore T

TABLE I (Con't)

Tabulation of Cases of Hemolytic Anemia Due to Drugs of the Para-Amino-Benzene-Sulfonamide Group

Reference	Sex Age	Total Drug	Total Days	Diagnosis	Lowest Hb. R.B.C.	Treatment	Outcome
Harvey Janeway	Female 26	12.2 gms. Sulfan.	2	Peritonsillar Abscess	18% 2,000,000 Dev.in 36 hrs	2 Blood Transfs.	Recovered
Harvey Janeway	Female 10 mos.	8.92 gms. Sulfan.	7	Mening. Meningitis	Dev.in 7 days	2 Blood Transfs.	Recovered
Kohn	Female 1 Yr.	6 gms. Sulfan.	6	Ot.Media	6.0 gms. 2,000,000	Intramusc. Blood	Recovered
Jennings Southwell-Sander	Male 45	30gms.sulfan. 6gms.pront. flav.	8	Mening. Meningitis	50% 2,500,000	Iron Transf.	Recovered

two million in 24 hours. Marked reticulocytosis, leukocytosis, bilirubinemia, urobilinuria, porphyrinuria, jaundice, and occasionally jaundice are also associated with this condition.

In considering predisposing factors, it was found that no one type of infection was associated with the acute anemia. The size of the dose and the concentration of the drug in the blood do not seem to be factors for both high and low values were associated with the syndrome. The carbon dioxide combining power in 19 of Wood's (106) 21 cases of acute hemolytic anemia cases was within normal limits. Table II is a table taken from a report by Wood (106) demonstrating a hypersensitivity of the red blood cell system to sulfanilamide after the four patients had recovered from a previous attack of acute hemolytic anemia. Case A.D. is a case cited by Willis.(105) All of the patients had fever.

Incidence. Acute hemolytic anemia is not more prevalent in one sex than in the other. It is, however, more prevalent in children than adults. Wood(106) has observed 522 patients treated with sulfanilamide. Of this group 144 were children and 378 were adults. A total of 21 cases of acute hemolytic anemia occurred in the children's group and 9 in the adult group, or 8.37% and 2.4% respectively. No large group of patients

TABLE

Table from Wood (106) Summarizing Five
Anemia Took Place Following a Second C

Patient	Sex Age	Diagnosis	First Course of Treatme: Days of Treatment	Total Dose	Dr %
No.1	Male 36	Acute Tonsillitis	5	21.9	
No.2	Male 4 days	Ophthal. Neo.	14	7.4	
No.3	Male 2	Pulmon. Tb.	7	14.0	
No.4	Female 7 Mos.	Otitis Media	3	4.5	
No.5	Female 9 Mos.	Pyelitis	3	5.8	

treated with sulfapyridine has as yet been reported. Hemolytic anemia, however, also occurs in patients treated with sulfapyridine.(29)(84) No hemolytic anemia associated with either prontosil or disulfanilamide has been reported but 2 cases treated with diamino diphenyl sulfone are reported.(62)

Course and Prognosis. The onset of the disease is from 24 to 72 hours after the beginning of the drug administration. The maximum anemia occurs from the third to seventh day, most often on the fifth day.

The mortality rate is low because the condition responds well to withdrawal of the drug and blood transfusions. Two fatal cases are reported, one by Wood (107) and the other by Koletsky.(52) (See Table I.) The case reported by Koletsky died 6 days after the beginning of the first dose of sulfanilamide and 2 days after the drug was discontinued. No transfusions were available. The death following the development of anemia reported by Wood (107) is evidently due to the fact that transfusions were started too late in the course of the disease. The drug had been discontinued on the sixth day but no transfusion was given until the twelfth day. The patient died on the sixteenth day after the drug had first been started.

Possible Mechanism. That the acute hemolytic anemia, which develops during the course of therapy with several drugs of the sulfonamide group, is due to an individual idiosyncrasy is very evident. That the condition does not occur in every case is one proof of this and the fact that 3 out of 4 patients have a recurrence of the symptoms when the drug is repeated is evidence that these patients are hypersensitive to the drug.(63) As with all idiosyncrasies, the mechanism by which it works is not understood. There is no bone marrow depression as is indicated both by the reticulocytosis and leukocytosis as well as by bone marrow studies that reveal a hyperplasia of both erythroid and myeloid elements. The drug does not hemlyze the red blood cells of these patients, nor are the cells of these patients more easily hemolyzed by hypotonic saline than in normal patients. (53)(106)(107) Histopathological study of other tissues, besides the bone marrow, reveals changes typical of any severe hemolytic anemia. The most typical finding is hemosiderin deposits in the tissue. So the pathology gives no clue to the mechanism of the anemia production. Both Long (63) and Wood (106) report the presence of porphyrin in the urine. Rimington (82) has found that porphyrin increases when sulfanilamide is given to patients. He believes that the porphyrin

production is not related to the bilirubinemia associated with red cell destruction, but he thinks it is produced because the sulfanilamide may possibly be responsible for a disturbance in pigment metabolism with a deep-seated effect upon the hemapoietic system. He says that hemoglobin forms bilirubin without passing through a porphyrin stage. It is necessary to conclude that the mechanism of the production of hemolytic anemia by drugs of this group is not understood.

Treatment. Treatment of hemolytic anemia is simple. Two main conditions must be fulfilled. In the first place, the drug responsible for the idiosyncrasy must be eliminated. This is accomplished by forcing fluids and discontinuing the drug. These drugs are excreted almost entirely by the kidney (68)(75) so water diuresis is very necessary. In the second place blood transfusions are necessary not only to tide the person over the critical period until the bone marrow is able to regenerate blood cells to replace those destroyed but also to prevent irreparable tissue damage in case of profound anemia. Many of these patients become so anemic that cyanosis is not present even though a considerable portion of the remaining hemoglobin has been changed to methemoglobin.(78) Furthermore, it is possible for toxic effects to manifest themselves from 3 to 4 days

after the last dose(18), and it seems too great a risk to take a chance and hope that the hemoglobin and red cells will not drop farther even though the drug has been discontinued. That these methods are satisfactory in preventing mortality is indicated by reports of Long (63), Wood (106)(93)(38)(74). Occasionally (63) if the infection is very severe and there is no control of it yet established when the anemia occurs, it is justifiable to continue the drug therapy provided the patient is carefully watched and repeated daily transfusions are given to control the anemia.

LEUKOPENIA AND AGRANULOCYTOSIS ASSOCIATED WITH SULFANILAMIDE AND RELATED COMPOUNDS.

Introduction

The literature concerning disturbances of the white blood cells due to the benzene-sulfonamide drugs is very much greater in amount than that relating of red blood cell disorders. Undoubtedly this is due to the fact that agranulocytosis is a very severe and often fatal complication following not only the para-amino-benzene sulfonamide group but also the benzene drugs, especially the benzamines, to which the group under discussion belongs.(56)

Agranulocytosis is, however, not the only disturbance of white blood cells encountered when the effects of these drugs on the leukocytes are reviewed Other conditions are leukopenia and hyperleukocytosis.

Leukopenia

Trumper in 1937 (77) in a letter addressed to the New England Journal of Medicine said that two cases which he had been treating with prontosil and prontolyn had had marked reductions in the total white count. One patient with a septic mastoid had dropped from 18,800 white blood cells to 4,000 with 35% granulocytes. The white count in the other patient suffering

from puerperal sepsis dropped from 20,000 white blood cells to 5,000 with 50%-60% granulocytes. This seems to be the first report which appeared in the literature which indicated that sulfanilamide and prontosil may affect the blood picture in some manner. In both instances the white cell count rose when the drug was discontinued.

Some believe that the derivatives of sulfanilamide as well as that drug itself have a depressive effect upon the white blood cells. This is the opinion of Bigler (7) who studied the leukocyte response of 33 patients to sulfanilamide therapy. Since they observed the number of leukocytes became less during the course of treatment, they concluded that the cell drop was an effect upon the cells and not a natural result of the termination or recovery from the original infection. That they made that conclusion seems especially strange when it is found during examination of their reports that no one type of cell was decreased out of proportion to the other cells. Although they did find that in three instances the total count was slightly below 5000, no evidence of granulocytopenia is present.

Britton (10) observed a group of 50 ambulatory patients who received the drug for two weeks, but whose blood counts were done 2 times a week, both during the

treatment and the following two weeks. Of this group 32 had no marked variations in white cell counts. In 14 cases the count dropped to between 5000 and 4000 and 4 cases had white cell counts that dropped below 3000. At the beginning none of the patients had either a leukocytosis or leukopenia. Blood counts were done on 17 untreated normal controls during the same period. The types of cases being treated consisted of cervical eriosions, urinary infections, gonorrhea, and one case of syphilis. Of their 14 cases whose count fell to between 5000 and 4000, 2 cases were lowest during the first week, 3 cases lowest during the second week, and 9 cases were lowest during the third week, that is, the first week after the medication had been discontinued. Those 4 cases in which the white cell counts dropped below 4000, had that occur during the third week. In only five of their cases was a true neutropenia observed where the granulocyte count dropped below 2000. After analyzing their findings, they found that there was a tendency for the average total white cell count to drop during the first week and remain low for the following two weeks with a rise to normal occurring at the end of the third week.

Two hundred and fifty cases treated with sulfapyridine were summarized by Lloyd, et al. (58).

They say that a small proportion of these patients had slight but definite decrease of the leukocyte count associated with a neutropenia and relative lymphocytosis. They observed no dangerous depression of the granulocytes and say that the white count quickly rose to normal levels with discontinuation of the drug.

Hodes, et al., (39) treated 71 cases of pneumonia with sulfapyridine. He reports 5 cases of mild granulocytopenia and one case of severe granulocytopenia which all recovered as soon as the drug was discontinued. Flippin (29) reports one case of leukopenia in a group of 100 cases treated with sulfanilamide.

From the reports of the results of sulfapyridine therapy it is apparent that a depression of leukocytes is associated with the administration of this drug. That the drug is an etiological factor concerned in the cell depression is evident since the count returns to normal after it has been discontinued. Whether these decreases in the total white count are in the nature of a mild hypersensitivity reaction or due to a direct toxic effect upon the white blood cells or marrow, we have no way of knowing. That either one of the two conditions may exist is possible. In every case the condition was mild and no other toxic symptoms were associated with the change in the blood picture.

In the 408 cases observed by Long (62)(63), no mild or transient cases of leukopenia associated with granulocytopenia are noted. The only white blood cell disturbances encountered was one case of agranulocytosis which developed during treatment at the hospital and one case of agranulocytosis which developed in an out-patient. Patients in their care who had leukopenia as a result of an infection recovered from this symptom as soon as the infection was brought under control either by sulfanilamide, sulfapyridine, or some related drug. Long and Bliss, therefore, conclude that these drugs have no direct effect upon the white blood cells except that occasionally agranulocytosis may occur associated with the administration of these drugs.

Reference to a case cited by Ives (42) has been made earlier in which agranulocytosis associated with a hemolytic streptococcic angina improved after sulfanilamide was given. Long (63) also has said that sulfanilamide is indicated in those cases of leukopenia and agranulocytosis associated with an infection, for the removal of the infection will remove the etiological factor. It seems, however, that this opinion should be slightly modified. Strasser and Singer (96) say that if any disease which is characterized by leukopenia is treated by sulfanilamide or its

derivatives the depression of the white blood cells is apt to increase to an even still greater degree. He cites a case of miliary tuberculosis treated with prontosil in which agranulocytosis developed. This was treated with nucleotide and the white count rose. This argues for the cumulative effect of the drug. Later a 3 day course of treatment with the same drug resulted in total disappearance of granulocytes from the blood. The count rose after the drug had been discontinued and a transfusion had been given.

Agranulocytosis

The first cases of agranulocytosis associated with sulfanilamide and prontosil administration were reported by Plumer (101); Borst (8); and Young (108) in 1937. Each of these cases terminated fatally. After these accidents had occurred, more reports were made of both severe granulocytopenia and agranulocytosis resulting when this group of drugs were given.

Etiology. Long (62) thinks that the agranulocytosis which occurs and is associated with therapy by sulfanilamide or other members of the group is different from the type of reaction seen in the cases of agranulocytosis or profound granulocytopenia associated with aminopyrine. He bases this opinion on the fact that often

patients who have recovered from the disease do not subsequently develop granulocytopenia or other symptoms when test doses are given, or therapy is reinstituted for other infections. Schwentker (92) also has observed a lack of hypersensitivity to these drugs when they are administrated.

Jennings and Southwell (84) believe the condition of agranulocytosis following sulfanilamide administration is similar to the condition which occasionally occurs following the administration of benzol, toluol, and their nitro and amide products. They remind us of the fact that sulfanilamide and its related substances are members of this group of drugs. With amido-pyrine, however, it is not known how much of the drug is apt to cause an attack. With sulfanilamide, however, it has often been observed that after 20 grams have been administered in a period usually over 14 days the blood disturbances of the white cells are apt to occur. The fact that damage to the white cells does not occur until after a large amount of the drug has been given for a long time causes Shecket and Price (93), Taub and Lefkowitz (99) to think that it is the cumulative effect of the drug given over a long period of time that finally wears out the myeloid tissue of the bone marrow. Since large doses do not affect all patients in the same

manner it would seem that only the organically deficient marrows are affected.

Allen and Short (1), McGuire and McGuire (70), and Jones and Miller (49) have been able to report sensitivity to the drug by patients after they had recovered from agranulocytosis after a course of sulfanilamide treatment and therefore regard it as a reaction of the hemapoietic system of a hypersensitive nature.

Again we mention another factor previously cited (96), that one should proceed cautiously with the use of prontosil if there is already evidence of some white blood cell dyscrasia. One case of agranulocytosis which developed in a patient that had miliary tuberculosis was cited. Coxon and Forbes (15) reported a case of typhoid which developed an agranulocytosis after the patient had been treated with sulfapyridine. Four cases of whooping cough with bronchopneumonia, which were treated with sulfapyridine and also developed agranulocytopenia are reported by Dolgopol.(24) All the diseases, tuberculosis, whooping cough and typhoid, are characterized by neutropenia and we wonder if the agranulocytosis which occurred may not have been influenced by the fact that changes already were present in the bone marrow before the drug therapy began.

Sex may or may not be a factor in the production

TABLE III

Tabulation of Reported Cases of
Granulocytopenia and Agranulocytosis

Reference	Diagnosis	Sex Age	Total Drug	Total Days	Onset of Diagnosis	Lowest W.B.C.	Treatment	Outcome
Borst	Pyelo-cystitis	Female 61	64 gms. Pront.	39	38 Days	960 1% Gran.	None	Died
Allen Short	Bartholin Abscess	Female 18	18 gms. Sulfan.	19	19 Days	2400 0 Gran.	None	Recovery
Alpert Forbes	Rheumatic Fever	Female 11	12 gms. Sulfan.	7		3200 0 Gran.	Liver Ext. Transf.	Recovery
Barnett et al.	Pneumoc. Peritonitis	Female 10	80.9 gms. Sulfapy.	26	3 Days Later	75 0 Gran.	Liver Ext. Trans.	Died. Hem. after drain. neck abscess
Berg Holtzman	Gonorrhea	Male 22	38.0 gms. Sulfan.	27	27 Days	1600 1% Gran.		Died

TABLE III

Tabulation of Reported Cases of
Granulocytopenia and Agranulocytosis

Reference	Diagnosis	Sex Age	Total Drug	Total Days	Onset of Diagnosis	Lowest W.B.C.	Treatment	Outcome
Bresgen	Pyelitis	Female 55	33.62 gms. Pront. & Sulfan.	21	21 Days	400 0 Gran.	Transf. Liver Ext. Nucleotide X-Ray	Died
Corr Root	1st Presc. by Osteopa.	Female 22	35 gms. Sulfan.	15		800 8% Neutro. 2% Eosin.	Transfs. Pentnucle-otide	Died
Coxon Forbes	Typhoid	Female 42	54 gms. Sulfapy	17		100 0 Gran.	Nucleinic Acid Calcium	Recovery
Culbreath Ellenton	Puerp.Sep. Malaria	Female 40	21.7 gms. Sulfan.	7	17 Days After last dose	700 0 Gran.	Transfs. Pentnucle-otide	Died
Cutler Crane	Salpingitis	Female			14 Days	1050 0 Gran.	Transfs. Pentnucle-otide	Recovery

TABLE III

Tabulation of Reported Cases of
Granulocytopenia and Agranulocytosis

Reference	Diagnosis	Sex Age	Total Drug	Total Days	Onset of Diagnosis	Lowest W.B.C.	Treatment	Outcome
Dolgopol Hobart	Pneumonia	Female 4	49 gms. Sulfapy.	14	14 Days	2000 0 Gran.	Liver Ext. Pentnucl. Transfs.	Died
Dolgopol Hobart	Pertussis Pneumonia	Female 1	27 gms. Sulfapy.	15	3 Days After last dose	2500 0 Gran.	Liver Ext. Pentnucl.	Recovery
Dolgopal Hobart	Pertussis Pneumonia	Female 18 mos.	14.5 gms. Sulfapyr	4	4 Days	4200 24% Gran.	None	Recovery
Evans Gaisford	Pneumonia	Child	No Record Sulfapy.	11	11 Days	1100 0 Gran.	- -	Recovery
Gray Adams	Pneumococcic Meningitis	Male 19	77 gms. Sulfan.	17	2 Days After last dose	2000 24% Gran.	None	Recovery
Hall	Pneumonia	Male 9	45 gms. Sulfan.	21	21 Days	2000 0 Gran.	Yellow bone Marrow Ext.	Died

TABLE III

Tabulation of Reported Cases of
Granulocytopenia and Agranulocytosis

Reference	Diagnosis	Sex Age.	Total Drug	Total Days	Onset of Diagnosis	Lowest W.B.C.	Treatment	Outcome
Hoffman	G.C. Arthritis	Female 19	49.2 gms. Sulfan.	18	3 Days After last dose	1350 0 Gran.	Transf. Liver Ext. Fe.Sulf.	Recovery
Trumper	Septic Mastoid	- -	- -	-	- -	4000 35% Gran.	Withdrawal of Drug	Recovery
Hohmann	Gonorrhea	Male --	24 gms. Streptoc. 7.2gms.Pront.	12	Drug discontinued because a drop occurred in the number of granulocytes on the 12th day. Decrease did not continue after drug discontinued.			
Ives	Septic sore throat with Agranulocy.	Male 52	- -	-	Agranulocy. Before Drug Given	1200 0 Granu.	Liver Ext. Pentnucle.	Recovery Soon as Drug Began
Johnston	Puerperal Sepsis	Female 23	61.3 gms. Pront. & Sulfan.	20	20 Days	1520 0 Gran.	Transfs. Pentnucle. 10 Tab.allowed	Recovery

TABLE III

Tabulation of Reported Cases of
Granulocytopenia and Agranulocytosis

Reference	Diagnosis	Sex Age	Total Drug	Total Days	Onset of Diagnosis	Lowest W.B.C.	Treatment	Outcome
Johnston	Puerperal Sepsis	Female 33	68.45 gms. Pront. & Sulfan.	22	22 Days	3500 0 Gran.	Transfs. Pentnucle. 5 Tab.Allowed	Recovery
Jones Miller	Gonorrhea	Male 26	over 30 gms.	20	20 Days	2300 5% Gran.	Yellow bone Marrow conc.	Recovery
Marcus	Pyelitis	Female 35	16.25 gms. Sulfan.	25	25 Days	1000 0 Gran.	Transfs. Pentnucle. Bone Marrow	Recovery
McGuire McGuire	Rheumatoid Arthritis	Female 35	27 gms. Sulfan.	30	30 Days	450 0 Gran.	X-Ray.Intra-Musc.Blood. Pentnucle.	Recovery. Sympt.Recur with Drug
Model	Rheum.Fever Endocar.	Male 20	54 gms. Sulfan.	18	18 Days	300 0 Gran.	Transfs. Pentnucle.	Died

TABLE III

Tabulation of Reported Cases of
Granulocytopenia and Agranulocytosis

Reference	Diagnosis	Sex Age	Total Drug	Total Days	Onset of Diagnosis	Lowest W.B.C.	Treatment	Outcome
Pearson	Sinusitis	Female 60	36 gms. Pront. Albun.	24	6 Days After Last Dose	600 2% Gran.	Tranfs.from Leukemia Patient	Died
Plumer	Subacute Bacterial Endocarditis	Female 54	43.25 gms. Sulfan.	23	3 Days After Last Dose	400 0 Gran.	None	Recovered
Sailor	Subacute Bact.Endocar.	Male 39	282.6 gms. Sulfan.	23	23 Days	200 0 Gran.	None	Died
Schwartz Garvin Koletsky	Chancroidal Ulcer	Male 32	56 Gms. Sulfan.	21	18 Days	300 0 Gran.	Pentnucle. Liver Ext. Transf.	Died
Schwartz Garvin Koletsky	Gonorrheal Arthritis	Male 57	66 gms. Sulfan.	13	13 Days	1350 20% Gran.	Pentnucle. Liver Ext. Transfs.	Died

TABLE III

Tabulation of Reported Cases of
Granulocytopenia and Agranulocytosis

Reference	Diagnosis	Sex Age	Total Drug	Total Days	Onset of Diagnosis	Lowest W.B.C.	Treatment	Outcome
Schwartz Garvin Koletsky	Erysipelas	Female 22	15 gms. Sulfan.	3	3 Days	2000 25% Gran.	Pentnucle. Liver Ext.	Recovery
Schwartz Garvin Koletsky	Puerperal Endometritis	Female 26	100 gms. Sulfan.	17	19 Days	400 0 Gran.	Pentnucle. Liver Ext. Transfs.	Recovery
Shecket Price	Pneumonia	Male 45	64 gms. Sulfan.	15	15 Days	50 0 Gran.	Pentnucle. Transfs.	Died
Shullenberger	Bronchopneu. Strep.Sore Throat	Male 44	52.39 gms. Sulfpy.	21	3 Days After last dose	1200 0 Gran.	Pentnucle.	Recovery
Sutherland	Spesis	37	52 gms. Sulfapy.	12	3 Days After last dose	1500 0 Gran.	Liver Ext. Pentnucle. Transfs.	Recovery

TABLE III

Tabulation of Reported Cases of
Granulocytopenia and Agranulocytosis

Reference	Diagnosis	Sex Age	Total Drug	Total Days	Onset of Diagnosis	Lowest W.B.C.	Treatment	Outcome
Sweeney	Sore Throat	Female 41	18 gms.	8	Had taken Previously ?	1800 18% Gran.	Daily Transfs. 7 Days	Recovery
Taub Lefkowitz	Pneumonia	Female 66	44 gms. Sulfan.	31	3 Days After last dose	1000 0 Gran.	Transfs. Pentnucle.	Died
Rosenthal Vogel	Recurrent Pneumonia	Male 1	15.25 gms.	?	During 4th Week	4000 10% Gran.	Liver Ext. Transfs.	Recovery
Rosenthal Vogel	Osteomyelitis	Male 10	95 gms. Sulfan.	14	17 Days	300 0 Gran.	Transf.	Died
Rosenthal Vogel	Pertusis & Bronchopneum.	Female 4	42 gms. Sulfan.	16	16 Days	1100 0 Gran.	Transf.	Died

TABLE III

Tabulation of Reported Cases of
Granulocytopenia and Agranulocytosis

Reference	Diagnosis	Sex Age	Total Drug	Total Days	Onset of Diagnosis	Lowest W.B.C.	Treatment	Outcome
Jennings Southwell-Sander	Ulcerative Colitis	Female 39	94 gms. Sulfan,	21	3 Days After last dose	444 0 Gran.		Recovery
Young	Rheumatism	Male 53	54 gms. Pront. Alb.	18	3 Days After last dose	1800 0 Gran.	Pentnucle.	Died

* * * * * *

of this condition. This is suggested since in the 43 cases listed in Table III, 26 are females and 15 are males. Two cases are not listed as to sex.

Possible etiological factors considered which may play a part in the development of the agranulocytopenia are bone marrow damage by previous infection, bone marrow changed by cumulative affect of long continued drug therapy, sex, abnormality of bone marrow of obscure origin, idiosyncrasy to drugs. That various factors may be concerned is evident, but the mechanism of the production of this abnormality remains unknown as in the case of other drug idiosyncrasy. A typical geographical distribution is not apparent in the case of this drug idiosyncrasy because the drug is so universally used and not confined so closely to Germany, United States, and Denmark as was the case with aminopyrine. Cases are also reported from France (79) and from other countries. The class distribution also does not hold for the sulfanilamide group because it is used for treating the infections of every class of people.

Pathology. The pathology of the bone marrow is reported in Table IV. As in agranulocytosis associated with other drugs, the findings vary.

TABLE IV

BONE MARROW STUDIES FROM FATAL CASES OF AGRANULOCYTOSIS

Bresgen	Decrease in myeloid cells. Increase in lymphocytes.
Dolgopol	Slight decrease in the nucleated cellular elements. Most cells immature. Prominence of reticulo-endothelial cells. Many lymphocytes and megakaryocytes.
Pearson	Many mononuclear cells. No segmented cells. Immature and developing erythrocytes.
Sailor	Hyperplastic and aplastic changes. Aplastic changes were most marked. No areas of erythrocyte destruction.
Shecket	Myeloblastic arrest.
Schwartz, et al.	Few mature neutrophils and erythrocytes. Maturation arrest with stem cell hyperplasia. Reduction of nucleated red cells accounted for the secondary anemia.
Taub, et al.	Hyperplasia of stem cells. Absence of mature cells of myeloid series.
Rosenthal, et al.	A decrease in the total cell count with a maturation arrest.
Young	Bone marrow aplasia.

Course of Disease. See Table III for information concerning reports of cases of severe granulocytopenia and agranulocytosis. The one symptom and finding associated most often with this condition is fever, which is an important warning signal of so many of the severe toxic effects of the sulfanilamide group of drugs. Fever usually precedes skin eruptions, toxic hepatitis, as well as agranulocytosis. Other warning signals which may or may not be an sign of beginning of this severe disturbance is a fever which continues even though the symptoms and signs of the original infection have disappeared, eruptions, gradual but slow decrease in the hemoglobin, and any abrupt rise or fall in the white blood count.

Table III is a tabulated record of available case reports in the literature. From a study of the cases we find that in addition to the fever associated with both the onset and the course of the disease there are chills, malaise, abdominal pains, sore throat, extreme toxicity, weakness, headache, confusion, and a foul breath. There is always leukopenia with profound granulocytopenia which may result in complete disappearance of all granulocytes. There may or may not be angina.

The reports of cases cited from the table indicate that the withdrawal of the drug does not always halt the progressive drop in white blood cells immediately

but the counts often continue to drop 2 or 3 days after the last dose. The count may not drop until a few days after the last dose is given. Often a steady but slow decrease in the total white count with no disturbance of ratio of different cells is a sign of the beginning of the dyscrasia. Twenty-six cases occurred in females, 15 in males. In two instances the sex is not stated. There is no predominence of any age group. No single type of infection predominates. The total dose of the drug ranges from 14.5 to 100 grams in a period of 7 to 39 days. With two exceptions the onset ranges from 14 to 21 days after the beginning of the drug's administration. Sulfanilamide, sulfapyridine, and prontosil had been used in various instances. Of the 43 reported cases, 18 were fatal.

Treatment. Treatment of white blood cell dyscrasias associated with drugs of the sulfanilamide

group is both prophylactic and active.

The best way to avoid the development of agranulocytosis associated with the para-amino-benzene sulfonamide drugs would be not to give the drug. Since that is impossible because these drugs are valuable aids in saving lives, it is necessary that the drug should not be given unless there exists an indication for its use. When it is used, the course of the patient's illness should be carefully watched. Fever, rash, slowly progressing anemia, slow response to treatment, gradual decreasing white blood count with or without neutropenia should all be regarded as warning signals. Other warning signals are development of jaundice, and any abrupt rise or fall in white blood cells.

Shecket and Price (93) say that if the efficacy of one of these drugs in combatting the infection is not demonstrated from four to seven days after the beginning of the administration, the drug should not be continued, for they believe the prolonged administration and the consequent large amounts of drug result in a cumulative toxic effect. Along this same line of thought is Bresgen's (9) advice to give large doses of the drug for a short time and therebye avoid the long continued effect of the drug upon the myeloid elements. He also

says that too small inadequate doses do not stop the infection but keep up a chronic low grade type of infection which results in added strain upon the bone marrow along with the effect of the drug. Shecket and Price (93) also remind us that the fact that the drug has been discontinued does not absolve us from continuing to watch the patient carefully, for often the agranulocytosis does not show itself until several days after the last dose of the drug. (96)

If there is already depressed or a changed bone marrow due to the nature of an infectious disease or other toxic factors, the question of whether or not to administer sulfanilamide should be carefully considered.

Carr and Root (14) advise that the physician and nursing staff be very watchful as long as the patient is on the drug, but especially so after 14 days have elapsed and the patient continues to receive it. Whenever a white count has been reduced to 3000 or 4000 the drug should be discontinued immediately and the patient carefully watched.(84) Hohmann (41) advises cessation of the drug thereapy whenever the granulocytes are decreased even though the total white count has not decreased.

After having considered the various suggestions

made which are all valuable in the prevention of serious blood dyscrasias, it is evident that it is very foolish to prescribe these drugs for every ache and pain. At this time the indications for the use of these drugs are well established both by clinical investigation and experience as well as by experimental work, and the medical profession contributes most to prophylaxis of severe toxic effects upon the blood by not using the drug ill-advisedly.

If agranulocytosis has occurred during or immediately following the ingestion of the sulfanilamide group of drugs, the drug must be discontinued at once if not already done. Elimination from the body is aided by forcing fluids. (68)(95)

Anemia is usually not associated with agranulocytosis. Blood transfusions should be made if anemia is part of the picture. The transfusions, however, do not stimulate the bone marrow and the relatively few leukocytes added to the circulation are of little or no help for they are rapidly used. X-Ray over the long bones has been suggested to depress the bone marrow but this has been abandoned as well as the theory that if sepsis or necrosis is induced the need for leukocytes will stimulate their production or maturation.(27)(43)(44)

Doan (23) says that nucleic acid exerts a

chemotactic effect upon normal myeloid foci and causes a prompt effective increase in the delivery of leukocytes to the peripheral vascular system. He does not believe that spontaneous recovery occurs from agranulocytosis because he thinks it is due to an intrinsic deficiency phenomenon. The nucleic acid and nucleotides supply the stimulus of maturation and the stimulus of initiation of development of cells to mature leukocytes if the bone marrow is in a condition to respond. (80)(81) If the condition has progressed to such a stage that the marrow has become aplastic, it will be impossible for it to be stimulated by any products of nucleic acid. Hall (35) suggests yellow bone marrow extract, because it supposedly supplies the intrinsic factor which is able to cause the bone marrow to produce mature cells and deliver them to blood. Yellow bone marrow extract is given orally in capsules containing 3.5 grains. Fifty to one hundred capsules are given daily until monocytes appear in the blood, and monocytes' reappearance in the blood stream are said to be of favorable prognostic significance. If adenine sulfate is used it is suggested that 1 gram be given intramuscularly three times a day until an increase in neutrophils begins. Jackson (43) advises pentnucleatide, 10 cc. intravenously four times a day. He has had very

good results with this substance.

From the cases reviewed, the best routine followed in treating agranulocytosis is to exclude the drug immediately and hasten its rapid excretion. Blood transfusion is necessary if the hemoglobin is less than 60%. Since it is not known what type of mechanism is responsible for the dyscrasia, it seems that no harm is done by using some form of a nucleatide or liver extract. The diet should be complete and the patient given good nursing care.

Hyperleukocytosis.

Before leaving the discussion of white blood cell disturbances, a few reports of hyperleukocytosis have been made. Fitzhugh (27) and Alpert (2) have found this condition to occur especially associated with drug fever and rash. A similar reaction has been noticed in laboratory animals.(64)

SUMMARY

1. A review of the literature concerning the disturbances of the erythrocytes and leukocytes of the blood associated with the administration of para-amino-benzene sulfonamide and its derivatives has been made.

2. Both a chronic slowly-developing hemolytic anemia and an acute hemolytic anemia are occasionally associated with the administration of these drugs.

3. Agranulocytosis and leucopenia are also associated with the ingestion of these drugs.

4. These hematological dyscrasias are very similar to the sensitivity reactions caused by amino-pyrine and other drugs of the amino benzene group of drugs.

5. Possible etiological factors which may be concerned in the development of these types of drug idiosyncrasy are size of the dose of the drug, the length of time the drug is given, the sex of the patient, the effect of the infectious process which is being tested, and the cumulative effect of the drug.

6. The actual mechanism of the production of the anemia is not known, nor is the mechanism of the production of agranulocytosis understood.

7. There is no typical characteristic bone marrow picture associated with these conditions.

8. The promiscuous use of the drugs of the para-amino-benzene sulfonamide group is to be discouraged. This applies to the medical profession as well as to the laymen.

9. Whenever the drug has been prescribed for a patient, the patient should be closely observed during the course of the drug therapy. If the response is not favorable, the drug should not be continued over too long a period of time.

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