Primary granulocytopenia

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PRIMARY GRANULOCYTOPENIA

SENIOR THESIS

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INTRODUCTION

Since the first recorded observations some thirty-three years ago, and more especially since the attention directed toward it by the work of Schultz in 1922, much has been written on the subject of granulocytopenia. In spite of the mass of literature on the subject, however, there is still considerable confusion existing as to the true nature of the condition. Indeed, some investigators even doubt its existence as a true disease entity, and there seems to be ample reason for this. As one reads over the published descriptions and case reports, it soon becomes evident that they deal with a group of diseases and not with a single disease. The group now includes almost any condition showing leukopenia and neutropenia, regardless of the cause or the clinical picture. The clinical features, including symptomatology, physical observations, pathology and treatment are abundantly described in the literature, but one usually must read many articles before all phases of the disease can be fully covered. Moreover, the present day textbooks and systems of medicine contain only brief discussions of the condition. The original nomenclature given to the condition was neither appropriate nor descriptive, and since that time, the new names that have been applied in the attempt to clarify the situation have only added to the general confusion. More recently, there have appeared reports of cases here and there that seem to occur after the use of the arsphenamines, the radio-active substances and roentgen rays, and certain of the coal tar derivative drugs that heretofore have been considered harmless, and have been used indiscriminately by members of the profession as well as by the general public.

In this paper an attempt will be made to correlate the
various manifestations of the disease and to arrive at a satisfactory nomenclature and classification. In addition a brief discussion of the etiology of the secondary granulocytopenia will be undertaken, with special reference to the action of the various drugs containing the benzene ring, to sepsis, and to the action of the radio-active substances.
DEFINITION

Primary granulocytopenia is a grave disease of unknown etiology characterized by a marked reduction in the total number of white blood cells and a great reduction in the percentage of granulocytes, accompanied by elastic, normal, or hyperelastic myeloid tissue. Following the peripheral neutropenia there may be any number of lesions and symptoms which might follow the removal from the body of such an important defense mechanism. The disease may be acute or chronic.(1)

NOMENCLATURE

The name agranulocytosis as originally proposed by Schultz (2) was an unfortunate one, but it has come into the literature quite prominently no doubt because of its brevity. It is incorrect. The name agranulocyte was originally chosen for the immature neutrophils without granulations seen in blood smears from cases of leukemia. By agranulocytosis is meant an increase in these typical neutrophils, which was not intended. Friedemann (3), being impressed by the usual severe localization in the throat, suggested the name angina agranulocytica. This name is also unfortunate as it implies that one is dealing with an infection of the mouth which causes neutropenia, instead of with a syndrome of unknown etiology. Moreover, it is now known that cases do occur without angina. Many other names have been suggested, such as: mucositis necroticans agranulocytica, sepsis with granulocytopenia, monocytic angina, and agranulosis; all of which are no better than the above. Baldridge and Needles(4) proposed the term idiopathic neutropenia, which is a good one. Schilling (5) suggested the name malignant neutropenia. Kracke (6) brought out the term granulocytopenia as more
correctly expressing the actual condition existing in the disease. It has the further virtue of being consistent with the accepted nomenclature describing variations in numbers and percentages of white blood cells, as proposed by Poerner (7), part of which is as follows:

- Neutropenia - a decrease in neutrophils.
- Lymphopenia - a decrease in lymphocytes.
- Granulopenia - a decrease in granular cells.

The last term is suggested as fitting the classification and expressive of the pathology.

**CLASSIFICATION**

Here again one runs into a divergence of ideas as to ways of classifying the condition. Kracke (6) has proposed a very logical classification from an etiological standpoint. Rosenthal (8), in his classification made according to the clinical manifestations and course, divided it into malignant (fatal) and benign (recovered) cases. Beck (1) further divided these into two classes as follows: (1) primary benign and primary malignant neutropenia, in which the etiology is unknown, and (2) secondary benign and secondary malignant neutropenia, in which the etiological agents are evident or in which one is dealing with examples of well recognized clinical entities.

In view of our present knowledge of the disease, this division into primary and secondary conditions seems to be the simplest and most logical. The further division into benign and malignant does not hold up so well in all cases. The classification proposed here is, with a few modifications, similar to that used by Piersol and Steinfield (9) and by Madison (10). The modifications used were influenced largely by Kracke's classification.
I. Primary Granulocytopenia - etiology unknown:

A. Acute - (Schultz type) in which the white cell count may fall to or below 2000, with 25 percent neutrophils or less. It is characterized by a somewhat sudden onset of lassitude, weakness, a tendency to sleep, and frequently the development of ulcers in the oral cavity or in any area of the body that is normally inhabited by bacteria. These bacteria may be non-pathogenic under normal conditions. It is fulminating, with the mortality varying from 90 to 100 percent.

B. Chronic - may be physiologic for the individual or may be a manifestation of broken compensation of the granulopoietic organ. It is characterized by weakness, easy tiring, tendency to fatigue, and chronic exhaustion; the severity of these symptoms being dependent upon the extent of decrease of the granulocytes. Roberts and Kracke (11) have shown, from a study of 8000 records from private practice, that the typical granulopenic patients are usually women between the ages of forty and sixty. It is also this type in which the acute type is most often seen, so they should always be regarded as potential candidates for the development of the acute attack.

C. Recurrent - characterized by acute attacks separated by weeks or months; the blood picture during the interim may show a normal picture or a chronic neutropenic state. This type has been emphasized by Kracke (12).

II. Secondary Granulocytopenia - etiology known:

A. Acute Infectious Diseases - associated with or followed by leukopenia and rarely near granulopenic states. This
blood picture may be due to an actual failure of the bone marrow to produce the normal number of neutrophils. These diseases include typhus, typhoid, measles, mumps, malaria, influenza, dengue, chlebotomus fever, Egyptian splenomegaly, and sometimes syphilis and tuberculosis. (10), (13).

B. Blood Diseases - exhaustion of the marrow as seen in pernicious anemia, in rare cases of acute leukemia, and in the terminal stages of chronic leukemia, aleukemic leukemia, aplastic anemia and some secondary anemias, splenic diseases, and malignant invasion of the bone marrow. (14), (15)

C. Sepsis - a neutropenia due to the effect of unusual bacterial toxins or to an unusual response of the bone marrow to ordinary bacteria. Usually sepsis is accompanied by an increase of the peripheral white blood cells and a great increase in the percentage of polymorphonuclear neutrophils. In any infection, however, the bone marrow may fail to respond adequately to the demand for leukocytes, and there ensues, gradually or rapidly, a more or less marked leukopenia. Coincidently younger and younger cells of the granular series make their appearance in the blood, and there are found in the smear polymorphonuclear neutrophils showing toxic degeneration, namely vacuoles in the cytoplasm and large deeply staining cytoplasmic granules... (16) It is probable that the large number of cases who give a history of oral infection prior to their attack of neutropenia had a depression of the bone marrow due to invasion by mouth organisms. Such cases have been
reported by Whitehead (17); Call, Gray, and Hodge (18); Kastlin (19); Thomas (20); Moore and Weider (21); Skiles (22); Talley and Griffith (23); and others. Often the infection is in the throat, but often enough elsewhere. Wherever it may be, its spread is facilitated by the fact that the body's resistance is markedly lowered owing to the destruction of the granulocytic cells. A vicious circle is set up; the farther the local lesion spreads, the more toxin is produced, the greater the bone marrow is damaged, and the less resistance is offered to the infection. It is true in some cases that the throat ulcerations are secondary to a primary bone marrow damage, as in leukemia, typhoid fever, and so forth. On the other hand, when we consider the marked intoxication produced by toxin generated by the pharynx in certain cases of tonsilitis, or diphtheria, it seems unnecessary in many other cases to look elsewhere than the throat for the primary source of toxin. Constitutional predisposition of the bone marrow to react to toxins with a granulopenia has been suggested as an explanation for these conditions. The fact that such a mild toxin producer as B. pyocyaneus apparently has been the causative factor would lend support to this view (24).

Of the many organisms isolated from the oral lesions and the blood stream of these patients, B. pyocyaneus been found a number of times and has been showed to have the power of producing a leukopenia in experimental animals (25), (26), (27), (28). Streptococci, staphlococci, and the Vincent's organisms have been found
frequently in these cases.(29), (30), (24). Fried and Dameshek(31) claim to have produced granulopenia in experimental animals with strains of b. subestifer isolated from human beings, and in rare instances a granulopenia has developed after injections of typhoid vaccine, showing that it may result from an overwhelming foreign protein reaction in a sensitive individual.(32), (33). Norris states that his recently isolated saccharomycete produces a marked leukopenia in laboratory animals.(34)

D. Radiation - in which the bone marrow is temporarily depressed following excessive dosage of radiation therapy. This happened in a case recently reported by Kracke(35). Waters(36) makes the pertinent comment regarding the effect of X-Rays on tissues "Small doses stimulate, large doses destroy." The effects are produced almost entirely by the action of the gamma rays. When the penetration is sufficient to reach the bone marrow and the entire body is exposed, all the effects described for benzene may be produced; but lymphatic tissues and tissues unrelated to the hematopoietic system are much more severely damaged than by benzene, while complete aplasia of the marrow is more difficult to produce. Local application of gamma rays gives a similar pathological picture in the area exposed, but the compensatory response in the unexposed tissue alters the hematologic picture. Exposing the spleen alone may decrease the total white cell count. True aplastic anemia rarely, if ever, results from the therapeutic or occupational exposure to roentgen rays
if even minimum modern precautions are observed, but radio-active substances are somewhat more dangerous because of the greater penetration of the rays. (37)

E. Chemical toxicosis - in which the neutrophils may entirely disappear for the blood stream following chemical poisons, such as benzene, arsenicals, and certain of the coal tar derivative drugs. Selling (38) first called attention to the peculiar selective action of benzene in depressing the hemopoietic apparatus. Benzol is known to produce both a neutropenia accompanied by symptoms of marked sore throat, as shown by Rohner, Baldridge, and Hansman (39), as well as purpura and aplastic anemia, as shown by the same authors and by McCord and Sweeney (40). McCord brings out the depressive action of benzene in an excellent case (41), and Kracke (42) has been able to produce experimental granulopenia in rabbits by the subcutaneous and intraperitoneal injections of small doses of benzene over long periods, in which the animals first developed a chronic granulopenia, this being followed by an acute attack with mouth ulcers, sepsis and death. The red cells and platelets were unaffected, and he concluded "that the smaller the dose of benzene, the more selective is its affinity for the myeloblastic tissues." Weiskottten and Steensland (43) obtained the same results, and Weiskotten later demonstrated further the toxic effect of benzene on the bone marrow as regards its capability of producing neutrophils. (44)

Although salvarsan was brought out by Ehrlich in 1910 and neo came into use in 1912, the first report of
their toxic action on the bone marrow did not appear until Evans(45) publication in 1916. Three years later Labbe and Lenslois(46) and Leredde(47) described other evidences of this depressive action. Farley(48), in 1930, collected reports of forty-six cases of depressed bone marrow function following arsphenamine therapy with a granulocenic period, and he suggested that the direct cause might be the disintegration in vivo of the arsphenamines, so that a benzol-like action could take place. This is the generally accepted theory now, although there is still some dispute. Lawson, Jackson, and Cattanach(49), in reporting twenty-eight cases of inorganic arsenic poisoning, have shown that inorganic arsenic may produce a marked diminution of polymorphonuclear leukocytes with a relative monocytosis. Wheelihan(50) has reported a case of granulocytic aplasia of the bone marrow following injections of potassium arsenite in a child. However, inorganic arsenic has been employed in medical therapeutics for centuries, but until the report of these cases the literature fail to reveal any cases illustrating its toxic effect on the bone marrow. Moreover, symptoms similar to those reported here are not given in pharmacological textbooks even as rare occurrences in inorganic arsenic poisoning. Prior to Evans report in 1916, no cases were found in the literature illustrating the toxic effect of organic arsenic on the bone marrow. There are other factors to be considered, however, for otherwise such reactions would occur more frequently. It is suggested that a predisposed, weakened hemopoietic
superatus is probably of great importance as a secondary factor in every case of bone marrow depression. Another possible factor is the syphilitic infection itself. There is at present no pathological evidence to indicate that these blood dyscrasias are due to actual invasion of the bone marrow by the spirochete, yet a syphilito-
toxic action should be kept in mind as a possibility. Stratton(51) suggests that the granulopenia may represent a form of Herxheimer reaction in which myelotoxic substances are liberated. He claims this to be more plausible because Meyer(52) reports a case following malarial therapy for syphilis. It is also a well known fact that patients who are not syphilitics, but who suffer from severe secondary anemia or even pernicious anemia are occasionally benefited by the empirical use of arsenicals. (51) In addition to the etiological factors mentioned above drug idiosyncrasies might be mentioned. This is supported by the fact that one form of arsenenamine may be rather well tolerated, but when one shifts to another form symptoms of toxicity may appear. (53)

More recently there have been numerous reports in the literature of the toxic action of certain drugs, especially amidopyrine and certain of the barbituric acid derivatives. Here again the benzene ring seems to be the toxic group. Madison and Squire(54) reported an analysis of thirteen consecutive cases showing that all of the patients were physicians, nurses, or patients under a physicians care and that they all had been using drugs containing a barbiturate combined with amidopyrine.
Three of these patients after recovery had an abrupt or fluctuating drop in granulocytes after medication. In two cases single doses of a benzene ring derivative caused such a release. Watkins(55), Grant(56), Hanch(57), and McGuire(58) all substantiate the observations of Madison and Squire. Since that time reports have been numerous indicating that amidopyrine is an important factor in the production of an acute attack of granulocytopenia. Holten, Nielson, and Transbol(59) described five fatal cases in patients who developed granulocytopenia after they had been hospitalized for other disorders and who received amidopyrine. In a subsequent report Madison and Squire(60) felt that all their fourteen cases received amidopyrine either alone or in combination with a barbiturate directly preceding the onset of the attack. Randall(61) and Hoffmen, Butt and Hickey(62) report similar findings. Attempts to produce this condition in rabbits with amidopyrine have thus far not been very successful. Madison and Squire(60) produced a drop and an antimortem disappearance of granulocytes in one rabbit given large doses of allylisopropylbarbituric acid (allonal) by mouth but failed in seventeen other rabbits to change the blood picture significantly. Hoffmen, Butt, and Hickey(62) obtained some depression in rabbits but they do not report any bone marrow studies in their preliminary report. The drugs most frequently mentioned in the literature are amidopyrine, allonal, dinitrolophenol and amytol compound.

I will now discuss primary acute granulocytopenia.
HISTORY

Apparenty there is little hope of finding early references to this condition in hematological literature, but it would be surprising if such a startling entity had not been described. In Morell Mackenzie's "A Manual of Diseases of the Nose and Throat" (1880), under the heading "Putrid Sore Throat", one finds a condition defined as follows: "Primitive gangrene of the pharyngeal mucous membrane, constituting an affection perse, and originating independently of any other malady, such as diphtheria or scarlet fever". (63) No doubt many instances of what today would be diagnosed as granulocytopenia were included in the group diagnosed as putrid sore throat. Brown (64) in 1902, described a case of "Acute Primary Pharyngitis with Extreme Leukopenia", in which the white cell count was 200. Turk (65) in 1907, described similar cases, although he did not distinguish them from the common leukopenia of overwhelming infection. Baldridge and Needles (4) have found a case of undoubted agranulocytosis in the records of the University of Iowa Hospital in 1910. Then in 1922 Schultz (2) described a number of cases which revealed an almost complete absence of granular cells associated with a group of symptoms and pathological conditions which he declared formed a clinical entity. The features he emphasized were a high fever, occurring generally in elderly women with necrotic throat infections, rapid exhaustion, slight jaundice frequently, leukopenia with few if any granular cells but no involvement of the red cells or platelets, and death within a short time in almost every case. In 1923 Friedemann (3) named the syndrome "agranulocytosis. After that cases were reported everywhere. In 1927 Kastlin (19) collected reports of forty-three cases and added two of his own.
At the present time the number of reported cases runs up into the hundreds.

**PHYSIOLOGY**

In order to obtain a more clear understanding of the blood picture in this disease one should be familiar with the mechanism of hematopoiesis. The granulopoietic tissue in the adult is located in the red marrow, which is to be found in the ribs, the vertebrae, the sternum, the bones of the skull, and the os innominatum. It is in the red marrow that granulopoiesis takes place normally. The subject of bone marrow as a hematopoietic organ means consideration of the entire marrow as a unit. It is an organ of no inconsiderable size, having been shown by Wetzel(66) to have a volume, in the adult human being, of 1,419 cc., which is thirteen times that of the spleen and almost equal to that of the liver. This whole structure will be found in a relatively uniform state under normal conditions; this means that the proportion of erythropoietic to granulopoietic tissue, as well as the proportion of the different levels of maturation within the two groups, is relatively constant. According to the studies of Doan and Zerfas(67) in three accident cases the ratio of erythropoiesis to granulopoiesis was 1:20, 1:16, and 1:5.5. Other authors have given a ratio of 1:3.(1) It can thus be roughly estimated that from three to twenty times more tissue is devoted to the production of granulocytes than to the production of erythrocytes, or, in other words, the volume of the granulopoietic tissue is from nine and one-half to twelve times that of the spleen. In contrasting this with the great excess of erythroid over myeloid cells in the blood stream, the reverse in ratio must be correlated with the greater length of survival of the erythro-
cytes in the blood stream. It would seem from this that the granulopoietic organ is manufacturing an extremely fragile and delicate product.

The circulation of the bone marrow is accomplished by the main nutrient artery and its accompanying veins, passing toward either epiphysis, and its capillary branches to the venous sinusoids, together with numerous anastomoses of this system with the small vessels of the bone along the shaft and with larger epiphyseal vessels in the mature bone. The capillaries that lead directly from the nutrient arteries of the third and fourth order, the "transition capillaries" of Doan, are not the functioning capillaries, as in other organs, but rather they lead into tufts of sinusoids. These sinusoids constitute the functioning vascular bed of the marrow; they have walls like capillaries and appear like them when collapsed, but when they are open have the diameter and lumen of large veins. The nature of these vessels was analysed by Minot(38), who introduced the term "sinusoid". When the sinusoids are dilated, there is a sluggish flow of blood, which brings the maturation factors to the right concentration for the white cells. Along the border of the marrow there is a narrow zone where the blood vessels of the marrow anastomose with those of the shaft of the bone. As granulopoiesis begins, every vessel of this marrow border is patent to the circulation; that is to say, there are no collapsed intersinusoidal capillaries. No other area of the marrow has so constantly a maximum supply of blood.(1)

Sabin(69) has shown that the granulocytes arise outside of the blood vessels and pass into them by their own motility. they develop around the patent, dilated sinuses or vessels of the hematopoietic tissue in the peripheral, vascular zone, and
and move toward their borders as they become more mature. The young granulocytes exist here in groups at the same stage of maturation and fill the sinuses. They are seen to move en masse against the walls of the capillaries until the wall is bent inward. When the stretching reaches a certain point, a leukocyte close to the wall flows in between two endothelial cells and the rest follow in rapid succession.(69)

The cell from which granulocytes develop (parent cell) is a subject on which there is a difference of opinion. According to Sabin(69), this primitive cell is a reticular cell found in the interstices between the fat cells of the marrow. There are three levels in the development of these cells:

**Level I:** This cell, with repeated divisions, gives rise to a primitive free cell. As the basophilia of the cytoplasm and the numbers of mitochondria of this free cell reach a maximum, the cell becomes a myeloblast. This is a period of growth and division, and the primitive free cell of small size is found in the largest numbers. By the process of basophilia and mitochondria the myeloblasts develop into promyelocytes, and this is level II.

**Level II:** With the changing chemistry of the granules of the cytoplasm of the promyelocytes, eosinophilic, basophilic, and neutrophilic myelocytes develop. This changing chemistry of the granules continues to the senile leukocyte stage. In level II, granulation increases as mitochondria and basophilia in the cytoplasm decrease. Cell division ceases in this level. Basophilia may be carried over into the cells of the circulating blood, especially in rapidly developing
leukocytosis. Sabin distinguished three stages in the development of the myelocytes according to their granulations, and designated them A, B and C; A is the youngest myelocyte and C the oldest myelocyte ready to develop into the metamyelocyte. When the myelocytes are fully formed, there is the onset of ameboid movements, and these cells are termed metamyelocytes. Ameboid movements may begin a little before the metamyelocyte stage or almost at the end of it. The metamyelocytes may appear in the blood stream, one percent being considered as normal by Schilling (5).

Level III: From the metamyelocytes are formed cells of approximately uniform size and content of specific granulations, and ameboid activity is at its height. These are the basophils, eosinophils, and neutrophils ready to be delivered to the blood stream. In level III, the cells show functional maturity with a uniformity in size. Division no longer takes place and polymorphism occurs. The leukocytes, according to Arneth (70), become mature in the circulating blood, involving nuclear changes, and finally pass into the nonmotile phase; the cells in this stage are the fragile forms with easily ruptured membranes seen in fixed films.

The rhythmic delivery of granulocytes from the marrow to the blood stream has been demonstrated by Sabin and her co-workers (71). Shaw (72) demonstrated that the afternoon rise is but part of a diurnal rhythm, with a second rise about midnight. This rhythmic delivery of cells persists in cases with leukopenia and leukocytosis, being particularly striking in the latter.
The approximate hourly delivery of cells to the blood stream suggests that the time for maturation from metamyelocyte to granulocyte might be expressed in units of hours or minutes, while the time for the maturation from promyelocyte through to metamyelocyte may possibly be expressed in units of days, probably from three to seven. With the concept in mind that bone marrow, as a hematopoietic organ, is the place where granulocytes are made, that it holds a large store ready for delivery and that its store of immature forms is small in number but with exceedingly high potentiality toward multiplication, growth and maturation, the question of the mechanism to maintain such a normal structure becomes the problem. Two different processes must be analysed: first, the mechanism of delivery of the cells to the circulation, and second, the mechanism of maturation.

The question of how great a part vasomotor influences play in the mechanism of delivery is uncertain, as experiments concerning the response of the marrow to nerve stimuli have not been satisfactory. (1) There is more definite information concerning chemotactic factors, however. Doan and his associates (73) were able to call the cells from the marrow by administrating inactivated typhoid bacilli, depleting the bone marrow of myelocytes and leaving only promyelocytes and myeloblasts. They suggested that it represented a chemotactic factor minus a maturation element. The cells of the marrow showed no toxic effect, and the marrow readily regenerated after the experiment. Of the many substances that have been shown to call leukocytes from the bone marrow, nucleic acid is most likely to be a part of the normal mechanism. Doan and others (74) studied the effects of large doses of nucleic acid. Their study of the bone
marrow showed clearly the chemotactic effect of the nucleic acid, with the massing of the leukocytes around the patent sinusoids. A marked diapedesis into the vessels and vacant areas of the marrow from which the granulocytes had been drawn occurred. They also found that the granulocytes could be called from the marrow by the split products of nucleic acid, the purine bases, adenine and guanine. Doan(75) later concluded that nucleic acid and its degradation products exert a chemotactic effect on normal myeloid foci with a prompt effective increase in the delivery of granular leukocytes to the peripheral circulation, under a controlled physiologic or rhythmic mechanism.

Of the maturation factors there is but meager knowledge, but those bacteria that produce a sustained leukocytosis introduce such a factor, as they produce an increased division, growth and maturing of the less mature leukocytes in the marrow far beyond the normal amount. Bacon(76) believes that even in infections the stimulus to a increased activity of the marrow comes from altered body proteins. The relationship of the degree of leukocytosis to the resistance of the body to infection is well known. Beck(1) ventures the theory that the resistance of the patient may not depend on the power of the granulopoietic tissue to respond, but upon the power of the tissues producing the maturation factor to respond. This has been shown to be true in regard to the erythrocytes in the case of pernicious anemia, in which a specific substance contained in liver is the maturation factor.(77) No doubt there is a similar factor in the body to regulate the normal production of granulocytes, although it has not as yet been found.

The function of the granulocytes is well known. Roberts and Kracke(13) have found that the mere loss of granulocytes
for seven days is incompatible with life. Granulocytes are one of the chief sources of immunity, and with their disintegration and released ferments, they give much active daily immunity to the body. They are thought to be the source of complement, which is in the plasma, owing probably to the continued disintegration of neutrophils. Complement is probably the most important single factor in the destruction of bacteria and the defense of the tissues. There is also some evidence to show that the granulocytes may be the chief source of supply for many of the various types of immune bodies, such as bacteriolysins, hemolysins and precipitans.

Weiskotten(78) has shown that the life span of the neutrophil in the blood stream of rabbits is about four days, and the cases studied by Roberts and Kracke(13) seem to prove that the life span of the human leukocyte is about the same. Some idea can thus be had as to the enormous numbers of granulocytes to be consumed daily by physiological degeneration. Cells in the nonmotile chase appear in the blood stream in showers, indicating that there is death of many granulocytes in the blood stream. Moreover, there are various physiologic overflows of granulocytes from the blood into the tissues, with consumption in the tissues, and elimination of neutrophils into the saliva, and probably into the entire digestive tract and onto all mucous membranes. There is apparently a granul loss of granulopoietic tissue with advancing years. Custer and Ahlfeldt(79) studied the tibia, the femur, rib, sternum, and vertebra in a hundred unselected cases and found that the cellularity of these marrows decreased with advancing years of life; the decrease corresponding in rapidity to the order named. The response of these marrows to a hematopoietic stimulus of a
given intensity is in the following order: vertebra, sternum, femur, rib, and tibia.

ETIOLOGY

EXCITING CAUSE - The exciting cause of primary granulocytopenia is still unknown. Schultz(2) first brought up the question as to whether the disease is primarily an unknown infection, resulting in bone marrow depression (this in turn followed by overwhelming infection) or whether an unknown chemical agent is responsible for the bone marrow failure to produce granulocytes. Friedemann(3) thought that the disease might be the result of an endocrine disturbance. Kastlin(30) suggested that the disappearance of the neutrophils was due to an increased peripheral destruction, an abnormal distribution of the cells or the failure of cell development. There is little or no evidence to indicate that increased peripheral destruction of the granulocytes is taking place. The spleen is seldom enlarged and the red cells are little disturbed. Roberts and Kracke(13) were unable to demonstrate a circulating agent which showed evidence of toxicity for the neutrophils, and the blood from patients with granulocytopenia has been injected into laboratory animals with no resulting disturbance of the blood picture.

There is also little evidence that the absence of the granulocytes is due to an abnormal distribution of the cells. Kracke(6) has checked both venous and capillary blood in his ten cases and the counts were essentially the same.

It has been well demonstrated, however, that the absence of granulocytes is due to a failure of cell development, since the bone marrow at autopsy has been found consistently poor in granular cell elements, with erythroblasts present to a normal
degree. Piette(81) has described these findings in detail.
Buck(82) and others have studied the bone marrow of the patient
during life, and they all agree that the bone marrow function
is depressed.

Assuming that it is primarily a dysfunction of the bone
marrow, the nature of the unknown substance responsible for
this depression must next be found. As has been pointed out
above, in the case of the secondary granulocytopenias, benzene
and its allied products, bacterial toxins, dead bacteria and so
forth may be responsible. In those cases designated as primary
granulocytopenia, however, the nature of the depressing agent
is still a matter of theory. A few authors believe that it
may be a form of allergy in which the bone marrow is the point
of least resistance. Schilling has produced a blood picture
similar to that of granulocytopenia experimentally in anaphy-
lexis, and there have been reports of cases following the
prophylactic vaccination against typhoid.(12),(33) A familial
tendency to diseases of the hematopoietic tissue has been
suggested as a possibility, but it has not been proven as yet.
Moreover, paralysis of the bone marrow, as expressed by some
authors, cannot be considered at this time owing to the lack
of knowledge concerning the regulation of the bone marrow by
a nerve mechanism.

The endogenous disturbance of production of chemotactic
and maturation factors is another theory. It is known that
two different processes might be considered concerning the
mechanism to maintain production and destruction of granulocytes
at a constant level: (1) the mechanism of delivery of the cells
to the circulation, and (2) the mechanism of maturation. It
appears from reports of sternal biopsies(8) and autopsies(33)
that one or the other of these processes might be at fault independently in different cases. In typical cases with myeloid aplasia, the part of the bone marrow that manufactures the granular leukocytes (level I) has ceased to function, or nearly so. When the manufacture in level I ceases and the supply in levels II and III of the bone marrow are exhausted, the granulocytes of the blood stream would totally disappear in from three to five days after the exhaustion of levels II and III. Thus it seems reasonable to suppose that the primary lesion is not in the bone marrow, but in the organ or tissue which gives rise to the substance (factor) that keeps maturation of the granulocytes regulated to a normal level or regulated so that production and destruction are kept constant. This has been shown to be the case in pernicious anemia and might easily be so in this case, although a maturation factor has not yet been found for the granulocytes as has been done for the erythrocytes. In the group of cases showing peripheral neutropenia with slight hypoplasia, normal, moderate or marked hyperplasia of the myeloid tissue, may be considered due to a lack of the chemo- tactic factor. The cells may be growing and maturing but are not being called to the circulating blood. It is apparently in these cases that the patients recover spontaneously or are benefited by the nucleotides. The normal stimulus for calling granulocytes from the marrow to the circulating blood has been shown by experimental work to be the liberated products (nucleic acid, adenine, guanine) from disintegrating leukocytes.

PREDISPOSING CAUSES - Granulocytopenia occurs chiefly in middle-aged women. Kracke(6) gives the incidence as being four times as common in women, and Hueper(84) gives the ratio as 3.5:1. Most of the cases occur in the fourth and fifth decades of life.
Cases have occurred in children (24), (25), in young adults and past the age of sixty (13), (26), but they are more rare.

The disease is apparently not contagious or infectious, though Hart(87) describes three cases occurring in the same family. No record of similar instances were found.

It has no seasonal variations but does have geographic limitations. In a review of the literature in 1932, Kracke(6) found that about eighty per cent of the cases had been reported from Germany and the United States, but only two cases from England. Reports from Italy were quite numerous, while there were three cases in Russia, one in Japan and one in the Dutch Indies.

It seems to occur in all classes of life and occupation, having been reported in school children, housewives, business and professional women, laborers, scrub-women, physicians, farmers, prostitutes, and ladies and gentlemen of leisure; it seems peculiarly prevalent in the last named class. For some reason the incidence of the disease is high among nurses and physicians. There was one doctor, one medical student and one nurse in the eight cases seen by Herkins(15), and one doctor and two nurses in the ten cases of Kracke’s(6). Logefiel(88) and Fisher(89) have made similar observations.

The disease is confined largely to the white race, although cases have been reported occurring in colored females(23).

PATHOLOGY

BONE MARROW - In practically all fatal cases the bone marrow is degenerated to a great extent. It is often liquid, due to the absence of the supportive elements of the granulocytes, and it varies from red to straw color. Normoblasts and megakaryocytes are present usually in their normal numbers, but the myelocytes
and polymorphonuclear cells are absent or nearly absent. There may be a few myeloblasts, some showing mitosis, but they are fewer than are present normally. In other cases, particularly with the maintainence of the gross fatty structure, myelocytes may be completely absent, or nearly so, but erythrocytes, lymphocytes and monocytes are present. The latter two types of cells may apparently be actually increased. The aplasia of the marrow involves the granulocytes only, and they are absent even under the oxydase reaction. Areas of patchy necrosis may occur in the marrow. Cases have occurred in which the marrow showed a normal, light or marked myeloid hyperplasia. It is therefore apparent that two types of pathologic change underlie cases of neutropenia. In one type maturation of granulocytes has ceased, and there are peripheral neutropenia and myeloid aplasia; in the other type, there is arrest of maturation and a normal or hyperplastic myeloid tissue.

Kastlin(19) removed marrow from the sternum during the height of the disease, in three cases, and showed "a cell-poor marrow with decrease in the granulocytic elements, the same as seen at autopsy". The hypoplasia of these granuloc cells is thus not a terminal event, and the decrease of the cells in the blood stream appears to be due to failure of development of the cells in the bone marrow". No bacteria have been found in the marrow, except those distributed throughout the body, and none of those were capable of reproducing the disease in laboratory animals(6).

LOCAL LESIONS - The peculiar local lesions are merely a consequence of the absence of circulating granular leukocytes; they are characterized by the absence of a surrounding inflammatory zone, since neutrophils are not available to form
The cellular reaction that does occur is comprised mainly of lymphocytic infiltration and the appearance of endothelial leukocytes, or macrophage cells. The lack of development of the protective inflammatory barrier to infection is the reason for the spread of such an infection. The most common site for the development of focal regions of infection and necrosis is along various parts of the gastro-intestinal tract, particularly at those points where bacteria are normally abundant. Lesions also occur in the vulva, vagina and cervix in the female. They vary widely in extent, ranging from a few small superficial ulcers to extensive gangrenous processes. They may be caused by the usual bacterial inhabitants, which gained the ascendancy as a result of the protective mechanism of the granulocytes having been removed. Edema is often present in varying degree in the cervical tissues.

LUNGS - The lungs frequently show subpleural hemorrhages with areas of pleural fibrinous exudate overlying small consolidated areas which contain red cells and perhaps bacteria, but no leukocytes.

HEART - In acute cases complicated by generalized infection, the heart may show the changes of toxic myocarditis. Bacterial emboli may be widespread.

LIVER - The liver may show changes incident to the septic processes, including cloudy swelling, varying degrees of fatty degeneration, and occasionally small multiple areas of necrosis. There is an increase of Kupffer's cells. There are bile casts in the bile capillaries and bile pigment in the hepatic cells. Sometimes there are interstitial lymphatic infiltrations.

Spleen - The spleen is enlarged, dark red and firm. The enlargement in some cases has been found to be due to a great
increase in the reticulo-endothelial cells, which outnumber the lymphoid cells. The lymph follicles are not prominent on cut surface. Microscopically, the sinuses are filled with erythrocytes, proliferating reticulo-endothelial cells and lymphoid cells. Oxidase-positive cells are absent or scanty. The lymph follicles are small and atrophic, and there are no young cells or lymphoblasts in the germinal centers, only mature lymphocytes. Small anemic infarcts are occasionally seen.

KIDNEYS - The kidneys show evidence of cloudy swelling, or may present the usual changes of acute nephritis due to bacterial toxins.

LYMPH NODES - The submaxillary, cervical, peribronchial and mesenteric lymph nodes are in general enlarged, and they sometimes contain hemorrhages. Microscopic examination reveals atrophy of the lymph follicles; there are no young lymphocytes in the germinal centers, only mature lymphocytes being present. There is a proliferation of the reticulo-endothelial cells.

Most of the above pathology was taken from the works of Beck(1), Kracks(6) and Jaffe(90).

SYMPTOMS

PREVIOUS HEALTH - A great many patients give a history of weakness or lack of vitality for varying periods before the onset of the condition, especially in the chronic recurring cases. Some patients state that there was a lack of energy for years and a marked weakness for a few weeks or months before the onset of the acute attack. On the other hand, neutropenia occurs in some patients who have always been well, and who were active physically and mentally until the sudden onset. It may be that an unsuspected granulopenia had been present in these cases. Occasionally the condition follows
in the wake of some other disease.

ONSET - According to Roberts and Kracke (13), the disease exists in the bone marrow before it appears in the blood stream and in the blood stream before it appears clinically. It has, therefore, three onsets: a marrow onset; a blood stream onset and a clinical onset. There is another type, however, with a blood stream onset and a clinical onset, the marrow being normal or hyperplastic (1). This is more rare. In Roberts and Kracke's case "on May 18 came the first gross drop on polymorphonuclears in the blood stream in the second attack. This was the blood stream onset. The marrow onset evidently came from three to five days previously when the marrow stopped manufacture. The clinical onset came, May 21, four days after the blood stream onset and six or eight days after the marrow onset. Counting the life of the leukocyte at four days, it would seem that, measured by the events of the second attack, the period of collapse occurred eight days after the marrow had ceased to deliver the finished products to the blood stream, and four days after they had begun to disappear from the blood stream.

SYMPTOMS OF BONE MARROW ONSET - Apparently there are no subjective or objective symptoms during this time. This onset may be acute or chronic, mild or severe, but there is no obvious change in the patient's health.

SYMPTOMS OF BLOOD STREAM ONSET - The symptoms in this stage are quite variable and seem to depend largely upon the patient. Some cases show weakness, easy fatigue, drowsiness and a feeling of apprehension. Headache, general malaise, slight fever and mental and physical collapse are sometimes seen. Other patients have vague or almost no symptoms during this period. The only constant and positive finding in this stage is the blood picture.
SYMPTOMS OF CLINICAL ONSET - The attack occurs suddenly and the outstanding feature is the extreme weakness and almost complete prostration. The temperature ranges from 101 to 106 degrees F., and is of the continual type. In mild or chronic cases the temperature may be normal or only slightly elevated. Headache, chills, vomiting and muscle pains are frequent, as is marked dysphasia and varying degrees of edema of the cervical tissues. Jaundice is present in about forty per cent of the cases, and Hueper and Garrison (91) reported gall bladder disease in three out of seven cases. There is usually no marked palor of the skin or mucous membranes except in some cases late in the disease. Inflammatory changes in the oral cavity of varying degree occur in the majority of cases. Ulceration and necrosis resembling diphtheria may involve tonsils, pharynx, gums, tongue, larynx, esophagus and more rarely the skin, rectum, vulva, vagina, or perineum. The ulcers are nonmargined with overhanging edges and a complete absence of surrounding inflammatory zone. The lesions may be so slight in some cases as to be easily overlooked. The liver and spleen are palpable in some cases, and the regional lymph nodes of the neck are usually enlarged.

CLINICAL TYPES-- 1. Acute, fulminating type, like the cases originally reported by Schultz. The onset is sudden, with a chill, high fever and necrotizing angina; occasionally there may be jaundice and albuminuria is frequently present. The blood shows an extreme degree of leukopenia and neutropenia, and the bone marrow reveals a widespread necrosis of the granulopoietic system. This type is rapidly fatal. 2. Chronic type, in which the illness is more prolonged. As described by Thompson (92), there are several days of fever, followed by a
moderate soft, tender enlargement of the lymph nodes and spleen, accompanied by moderate to extreme leukopenia and a reduction in neutrophils, often reaching an almost complete absence. Toward the end of the disease the pharynx becomes red and sore, and scattered, round, superficial, whitish soots appear on the posterior pharyngeal wall. The disease lasts from one to three weeks, and the patients usually recover. Krecke(6) has shown that there also exists a chronic type of neutropenia that may never show evidence of an acute attack. The patient may have a white cell count as low as 1000, with neutrophils almost or entirely absent from the peripheral blood, and the average white cell count may range around 2000 to 3000. 3. Recurrent or relapsing type, in which there are two or more attacks several weeks or months apart. The symptoms during an attack may be similar to those in the fulminating or subacute types. The blood picture in the interim may be normal or show a chronic neutropenia. Death may occur in the second or third attack, the patient may make a complete recovery, or the process may become chronic.

LABORATORY FINDINGS - The findings of greatest diagnostic value are those of the blood, in which there is a marked neutropenia, with later involvement of the lymphocytes and monocytes. In a severe, fulminating case the total leukocytes may be so low as to be practically uncountable; in the mild cases with a relatively slow course, the count may be normal at first, but may gradually fall to 1000 or less per cubic millimeter. The entire drop in the leukocyte count at the beginning of the disease is due to the disappearance of granulocytes. In fulminating cases, when first seen, the total leukocyte count may be 700 or 800, or even as low as 100
or 200. In practically all cases the counts are below 2000. The neutrophilic granulocytes may range from around 40 or 30 per cent in the most chronic and less severe cases to a total absence in the fulminating cases. Their morphology, in practically all cases, is normal, but immature forms may be present. Lymphocytes may at first be present in their normal absolute numbers, but as the disease becomes more and more severe there is a relative increase, but in most cases an absolute decrease, of lymphocytes. They may become markedly reduced, but their morphology remains normal. Monocytes are also present normally at first, but as the disease progresses, there seems to be a depression of the reticulo-endothelial tissue, so that the absolute number becomes much reduced. In typical cases the erythrocytes and hemoglobin are not affected, unless the disease lasts longer than the usual ten days; in which cases a secondary anemia may develop. The blood platelets are usually normal or increased in number. They may become reduced if the disease is of fairly long duration and the bone marrow becomes more or less aplastic, but usually the patient dies before this eventuality occurs.

Many cases have shown positive blood cultures; streptococcus viridans and hemolyticus, staphlococcus aureus, pneumococcus, bacillus coli and bacillus pyocyanus being found most frequently. Their occurrence in the blood stream is probably due to an early breaking down of the normal protective mechanism of the body. Cultures and direct smears from the throat and mouth usually show only the organisms that may occur in any oral lesion.

The urine usually shows albumin, occasionally hyaline casts and often bile.
DIAGNOSIS

A typical history is the appearance of sore throat in a middle-aged patient, usually debilitated, accompanied by chills and fever, ulceration and then membrane formation in the throat and on the buccal mucous membrane. This picture may vary widely, however, and the diagnosis can be made only on the low total white cell count and the neutrophenia. Acute follicular tonsillitis, Vincent's angina and diphtheria are to be differentiated by appropriate clinical means and the absence of leukopenia. Streptococcic sore throat with septicemia occasionally gives a leukopenia, but rarely less than 4000 cells and the differential count usually shows at least 35 per cent neutrophils. In typhoid fever, influenza and the other diseases producing leukopenia the total white cell count is rarely less than 4000 cells, and the neutrophilic percentage is a least 25 per cent. Generalized, rapidly advancing tuberculosis may show a leukopenia as low as 3000 cells, but the neutrophils comprise from 90 to 99 per cent, mostly immature forms.

Primary granulocytopenia must be differentiated from the secondary form, and careful inquiry should be made regarding intoxication from benzene, the administration of arsenicals, especially those of the arsenphenamine group, and the use of the roentgen rays and radium.

LYMPHOSARCOMA - This condition may cause leukopenia with a reduction in neutrophils. The clinical course will serve to make the diagnosis, and it may be confirmed by biopsy.

LEUKEMIAS - Acute aleukemic leukemia may cause particular difficulty in diagnosis, but the severe anemia, hemorrhagic diathesis, and presence of myeloblasts or lymphoblasts in this disease are evidence against granulocytopenia. In rapidly
developing acute leukemias the leukocytes may fall to 2000 or
below, and the differential diagnosis may be very difficult.
Biopsy of the bone marrow will establish the diagnosis in
doubtful cases.

APLASTIC ANEMIA - In aplastic anemia, the onset of the fever
is usually not so abrupt, and there are subcutaneous hemorrhages
and hemorrhages from mucous membranes. There is aplasia of the
bone marrow as a whole, resulting in a yellow marrow which is
completely barren of erythrocytes, leukocytes and megakaryo-
cytes. There are leukopenia, neutropenia, thrombocytopenia
and rapidly advancing anemia of the hypochromic type. The
color index is irregular, and there is a relative increase in
lymphocytes.

INFECTIOUS MONONUCLEOSIS - In this disease there occasionally
may be low leukocyte counts. The clinical manifestations are
usually much less severe than in benign and malignant
neutropenia, and an injection of foreign protein will result
in an increase in the circulating neutrophils.

PERNICIOUS ANEMIA - Care must be taken to distinguish pernicious
anemia in an aplastic phase and metastases to the bones
producing the so-called myelophthisic anemia. If anemia is
present in granulocytopenia, it is of the secondary type; the
erthrocytes are more or less achromatic and their average
diameter is less than seven microns.

PROGNOSIS

At first the mortality of granulocytopenia was thought to
be 100 per cent (2). Kastlin (19) in 1927, reported a mortality
of 95 percent, while Friedemann (93) in the same year, gave it
as 92 percent. Harkins (15) in 1931, reviewed the literature
and collected one hundred and fifty cases with twenty-seven
recoveries, the approximate mortality rate being 82 per cent. Kracke(6) reported a mortality of 85 per cent in two hundred and fifty cases he reviewed. Taussig and Schnoebelen(94) in a review of three hundred and twenty-eight cases (which included the secondary types) found that the mortality was 75 per cent without special therapy and with miscellaneous forms of treatment; 63 per cent with blood transfusions; and 53 per cent with roentgen treatment. Jackson et al(95) reported a mortality of 30 per cent in fifty-four typical cases treated with the nucleotides. As can be seen from the above, the reported mortality rate has decreased steadily since 1927, and the increasing use of blood counts will probably cause the reported rate to continue to decrease, as the more chronic cases of neutropenia are added to this group. The fulminating cases are rapidly fatal, while recoveries are common in the milder and more prolonged cases. Rosenthal(96) has stressed the point that, in patients with total counts below 1000, death will likely occur, while in those with counts above 1000, the chance of recovery is good. This observation was based on five deaths and five recoveries in his ten cases. However, Wyatt(97) reported a white cell count of 700 with recovery, and Call, Gray and Hodges(18) reported one of 640. Kracke(12) reported a case in which the leukocyte count fell to 470 and the patient recovered, only to die in a second attack. Recoveries and deaths are reported with all forms of treatment, and a great many patients have recovered without any active treatment. It can thus be seen that the reduction in mortality cannot be ascribed solely to any one mode of treatment, but is more probably due to the recognition of more cases, owing to the more critical studies of the blood and the inclusion of the more
chronic cases in the group. Some authors consider that an absolute increase in endothelial leukocytes has some relationship to the severity of the disease and its prognosis, but there is not sufficient evidence in the material collected from reported cases to make this a certainty. (1) In the early stages of the disease, the outcome cannot be determined, as in the cases showing myeloid aplasia, it depends entirely on whether maturation of the granulocytes will be resumed. In the cases showing peripheral neutropenia and normal or hyperplastic myeloid tissue, the outcome depends upon the resumption of delivery of the cells to the circulation. Finally, it is well to bear in mind the tendency of the recovered patients toward remissions. If final reports on these cases were available, it is probable that the mortality rate would be appreciably increased. (6)

TREATMENT

A review of the literature soon shows that a specific and satisfactory treatment for granulocytopenia has not yet been discovered. Indeed, many authors believe that recovery, when it does take place, is spontaneous and not influenced by the type of treatment used. There are many records of spontaneous recovery taking place in what seemed in the beginning to be a malignant (fatal) case. In many cases in which recovery occurred, several types of treatment were used, so that it was impossible to draw decisive conclusions as to the efficacy of the measures that were used, or to say which, if any, was responsible for the cure.

The treatment of granulocytopenia should be considered in two parts. First, treatment in the acute cases, which means the employment of an agent that will stimulate maturation of
The granulocytes and cause their delivery to the circulating blood in the shortest time possible. The mere absence of granulocytes from the blood stream for seven days is probably incompatible with life, and in a system devoid of granulocytes infection quickly takes place. Second, the treatment of patients with chronic disease with the object of preventing a recurrence, or an acute attack, if possible.

PREVENTIVE TREATMENT

There is little definite information available as to the management of chronic cases and the prevention of recurrence of acute attacks. Roberts and Kracke(11), in a study of 3000 records of leukocyte and differential counts in a series of ambulatory patients, found that one out of every four had granulopenia. This is significant, since the acute attacks develop chiefly in patients with a chronic neutropenia. The evidence accumulated indicates that weakness, exhaustion, fatigue and a tendency to sleep are the chief symptoms of a depressed granulocyte count, although some patients give a history of great mental stress or emotional shock. Patients who have had more than one or two attacks know their feelings when granulopenia is present. A number of these patients with chronic granulopenia improve with rest. Roberts and Kracke reported one patient who improved during 1931, when business was so poor that he rested more than usual, and another patient who improved after retirement. Granulocytopenia is known to affect the heart, many cases of arrhythmia, tachycardia, and myocarditis being reported, so it appears that rest in bed and freedom from mental and emotional stress is advisable in these patients, until the granulocyte count approaches normal. It is also advisable that these patients should refrain from using
any drugs known to have a depressing effect on the granulopoietic tissue, such as those containing the benzene ring. An attempt should be made to clear up all foci of infection, and particular attention should be paid to general, and especially oral, hygiene. It is believed that diet may play an important role both in etiology and treatment. Doan(93), experimenting with pigeons by underfeeding, reduced the red marrow of the radius to extreme hypoplasia, and there were only three component parts left: blood vessels, fat cells and a minimal residual framework of reticulum and reticular cells. Lossen(99) made counts of the total number of cells per cubic millimeter in children's marrow, finding that their number seemed, in a general way, to be dependent on the state of nutrition. If this can be proved and the dietary factor that has a specific influence found, it will have a most practical bearing on the treatment of the disease. Minot has suggested that a diet high in vitamin B is of material aid in the chronic and recurrent types. Operative procedures should be approached with considerable caution on these patients, as the granulopenic state may develop at any time and prevent proper healing. All patients having a granulopenia should be under careful observation, and frequent blood counts should be made.

ACTIVE TREATMENT

Treatment of all the granulopenias, secondary as well as primary, if the clinical manifestations warrant, has two principal objects: to stimulate granulopoiesis and to control infection. For the stimulation of granulopoiesis there have been several methods advocated, the relative merits of which are now being determined.
TRANSFUSION - The oldest method of stimulating granulopoiesis is the transfusion of small amounts of whole blood - not over 300cc. Even though the mortality in cases in which this method has been used alone is 64 per cent and though there is much antagonism to its use, some authors believe that a few transfusions in the fulminating cases may have some value. (6), (10). The use of transfusions is essentially empiric and no definite conclusions can be drawn from the literature as to its efficacy, as other measures were used along with it in practically all the cases. Sabin (69) has shown that repeated transfusions will lower the rate of erythropoiesis, and it may perhaps lower the granulopoiesis as well. Moreover, there is no actual evidence that transfusions actually stimulate granulopoiesis or that the neutrophils added in a few hundred cubic centimeters of blood would be of any assistance, as the life of the neutrophil is so short (5 days) and the number added would be so small compared to the number needed. Indeed, there are reports of cases in which the transfusions seemed to be followed by a reduction in the white blood cells. (100), (101). One of the patients treated by Jackson et al. (101) was given a transfusion after neucloptides had caused an increase in white cells to start, and there followed a reduction in white cells. Such a reduction of white cells was not seen in other cases treated with neucloptides alone, after a rise had started. As Beck (1) has pointed out, the blood from a healthy donor may contain the principles to bring about normal maturation of the granulocytes, but the amount of this substance received by the recipient would be very small compared to the amount needed. However, Madison (10) believes that whole blood may be of some value in preventing the entrance of infecting organisms into
the blood stream.

IMMUNOTRANSFUSION - Fisher(89) was the first to transfuse the blood of a recovered patient in the treatment of granulocytopenia. Chrisman and Hinton(102) have reported three cures in as many patients by the same procedure, and Harkins(15) has reported a similar case. However, in all these cases other therapeutic measures were used along with the transfusions, and in most cases, improvement occurred at about that time in the course of the disease that improvement is to be expected in cases with spontaneous recovery. Thus no definite conclusions can be drawn from these cases. Beck(103) points out that "there is not any evidence so far to indicate that any kind of immune bodies are formed in the blood stream in this disease. If a recovered patient's serum contained immune bodies, releases and recurrences should not be the rule. One attack does not give immunity from further attacks. It does not seem advisable to put a strain on the blood forming tissues of any patient who has had an attack of such a fatal disease. We would not use a patient who has pernicious anemia as a donor for a recipient who had the same disease."

ARSPHENAMINES - Arsenamines and neo-arsonamines have been used in the treatment of granulocytopenia, either alone or added to the transfused blood. In view of the recent knowledge of the depressing action of these substances on the bone marrow, this practice does not seem wise. Farley(48) has collected thirty-nine cases from the literature in which the function of the bone marrow was depressed following the use of various preparations of arsphenamine. The symptoms varied from those of purpura hemorrhagica to those of severe aplastic anemia and malignant neutropenia, depending on whether the principal effect
was on the granulopoietic, megakaryopoietic or erythropoietic tissue, or on all of these combined. In as much as these substances contain the benzene ring, which is a known depressant of the bone marrow, it does not seem wise to use such substances in a disease characterized by a depressed bone marrow function.

SPLENECTOMY - Splenectomy was performed in one case reported by Baldridge and Needles(4). There was not the usual rise of leukocytes after operation, as in other cases, but there was the usual platelet response. The patient died thirty-five days after operation. The bone marrow at autopsy showed an overgrowth of myelocytes and myeloblasts almost as marked as in myelogenous leukemia. However, as a specimen of the bone marrow was not obtained before the splenectomy, the part played by the splenectomy in this overgrowth of myeloid cells could not be determined.

IRRADIATION OF THE BONES - Another method of stimulating granulopoiesis is the irradiation of the long bones. The first reports of the results of irradiation were made by Friedemann (93) in 1927, with improvement in the four cases so treated. A year later he reported the cases of two additional patients who survived, as well as of four which resulted fatally; three of the latter dying within twenty-four hours following the first treatment, the fourth developing a pneumonia with septicemia. He applied, in measured amounts, 1/20 of an erythema dose of roentgen rays to the bones of the skeleton, using a hard filter (6 millimeter copper). He irradiated the long bones, giving from one to three treatments at intervals of from two to several days. He reported that improvement may begin within twenty-four to thirty-six hours, both in the symptoms and blood picture.
He stated that no definite judgment could be rendered as to the dosage; one irradiation with 1/20 skin units dose may produce a blood crisis in one patient, and in another it may take three doses to produce a change in the blood picture.

More recently Friedemann(105) has reported a series of forty-three cases treated exclusively by roentgen irradiation, with the exception of a few isolated cases in which a few ineffectual injections of Yatren-Kesein were given. Twenty-three of these patients died from complicating sepsis and five within thirty-six hours after irradiation; Friedemann does not feel that these should be considered in the evaluation of roentgen therapy.

Of the remaining fifteen uncomplicated cases, thirteen were reported as cured. Improvement in the blood picture in those surviving was usually noted within twenty-four to thirty-six hours after irradiation. Waters and Eiron(36) have reported five cases treated with roentgen ray; four are still living, one was in extremis when first seen. In their cases, the lower extremities, the pelvis, upper humeri, and shoulder girdle were irradiated in the course of three or four treatments. Voltage factors of 200,000 with aluminum and copper filtration, both separate and combined, with effective wave lengths ranging from 0.197 to 0.161 angstrom units were used.

The measurement of the dosage was effected by the use of a dosimeter, reading in roentgen (R) units, adopting 600 units as the erythema dose. When such a small dose is to be used, an accurate determination is imperative. It is known that roentgenotherapy may, under certain circumstances, provoke a granulocytopenic syndrome, so care must be taken to stay within the prescribed dosage. Neidhardt(106) reported the treatment of a severe case with irradiation, using 1/20 of an erythema dose.
Two hours after the treatment the leukocytes rose from 500 to 800; eight hours later 1700; the second day 3800. A second treatment was given, and eight hours later the total cell count was 9200. The granular cells increased, and the clinical condition improved.

Waters and Firor (36) remark that "the original selection of 1/20th of an erythema dose was both fortuitous and fortunate. Proceeding on the basis of the so-called Arndt-Schultz law that 'small doses stimulate, large doses destroy', it was decided that a dosage which was likely to produce a biological effect without exceeding the stimulation limit was naturally the one to be used. Following the application of this dose, it was found that in healthy adults there was also an increase in reticulocytes following such bone marrow irradiation. Thomas later reported a not inconsiderable increase in the leukocytes in leukopenia resulting from typhoid fever when 1/20 of an erythema dose was used. One cannot be sure whether this apparent stimulation is the result of chemical changes in the blood or due to the ray itself. There is no evidence that a cell cell per se is affected by the roentgen ray other than in a destructive manner. So far as we have been able to determine, those who have adopted this method have all used 1/20 of an erythema dose. Voltage factors have ranged from 130,000 to 200,000; filtrations of 3 mm. aluminum, 3 mm. aluminum, 1 mm. aluminum plus 0.5 mm. copper, and 0.6 mm. copper alone have been used. It is interesting to note that Friedemann's patients began to show improvement following a single treatment with the use of a 0.5 mm. copper filter, whereas aluminum filtration often required three treatments to produce a similar effect. The total quantity of radiation administered is usually
divided into three or four small doses."

The question as to the rationale of irradiation of the long bones has not been definitely settled. It is true that in the adult the marrow in the shafts of the long bones is mostly adipose tissue having little or no blood-forming function. Yet it is equally true that, when excessive or pathological demand exists, there is a formation of new centers by differentiation of the myeloblasts into granular myelocytes, the adipose tissue of the bone marrow being replaced by this newly-formed tissue. It is possible that irradiation may aid in the formation of this new tissue. Furthermore, the cancellous portion of the long bones and some of the flat bones are included in the fields of radiation. The only way of proving just what effect, if any, that roentgen ray has directly on the yellow marrow would be through biopsy as soon as the young granulocytes were appearing in the blood stream. Some say we cannot be sure whether this apparent stimulation is the result of chemical changes in the blood, or due to the roentgen ray itself, or whether the recovery is spontaneous. As stated above, there is no evidence that the cell per se is affected other than in a destructive manner. Friedemann(93 ) says:"It is evidently essential that as large a surface of the body as possible be subjected to the irradiation. Whether, thereby, a direct effect is exerted upon the bone marrow, or an indirect effect by the irradiation of other tissues is also an open question." It has also been suggested that the effect may be due to the stimulation of the organs of internal secretion, with the production of maturation products for the granulocytes. Doan(75 ) believes that the rays benefit the type of case showing peripheral neutropenia with normal or hyperplastic myeloid tissue. The roentgen rays in
these cases bring about a primary destruction of some of the
intact myeloid foci with a liberation of autogenous nucleotide,
which then initiates the maturation and delivery of granulo-
cytes from the remaining myeloid foci. It appears, from a
review of the literature, and following the results in the
cases treated by Cell at al(18), that roentgen ray might have
an important role in the stimulation of the granulopoietic
tissue, but definite proof is lacking.

NUCLEOTIDE THERAPY - The most recent method, and probably the
most correct, physiologically, is the use of adequate amounts
of pentose nucleotide. Nucleotide in its strictest sense means
the combination of nucleic acid with a base. The sodium salt
of nucleic acid was used as a therapeutic agent to stimulate
leukopoiesis thirty years ago and enjoyed considerable favor
for a time, but was subsequently forgotten.(10) But when
Jackson(107) demonstrated in 1924 the presence of considerable
amounts of a nucleic acid derivative in the normal blood stream,
principally in the nuclei of the white blood cells, the
probability that it was an important part of the physiological
stimulus for the formation of the granular cells was apparent
and interest in it was renewed. He described this substance as
being "a complex molecule made up of two pyrimidine and two
purine nucleotides linked together. These nucleotides are in
turn made up of a purine and pyrimidine base together with
pentose and phosphoric acid. These compounds may be broken
down further to nucleotides with a loss of phosphorus acid and
still further to the purine or pyrimidine bases with a loss of
pentose". Doan(75) was the first to show the chemotactic effect
of nucleic acid. He found that after the administration of
nucleic acid, there was a leukopenia preceding the leukocytosis,
due to a temporary storage of the granulocytes in the spleen. Then he found that the granulocytes could be called from the marrow by the split products of nucleic acid, adenine and guanine, and that after giving these substances, the leukocytes were not withdrawn from the circulation by the spleen, so that there was a direct leukocytosis without the temporary leukopenia. Reznikoff (108) reported four cases of malignant neutropenia, three of which recovered following treatment with nucleotides. He used the purine bases, adenine and guanine. He later showed that these substances given intravenously to rabbits caused a marked increase in the number of neutrophilic granulocytes without any effect on the temperature or on any other cells. Jackson et al. (101), in their series of cases, used the unbroken pentose nucleotide, called nucleotide K-96, which is prepared by Smith, Klein and French company, according to the technique of Jones and Perkins (109). In a series of twenty cases of granulocytopenia of varied etiology fourteen of the cases recovered. Two of these cases had relapses after an interval of a few days, but they again responded favorably to treatment. One other showed improvement but died of pulmonary hemorrhage after the blood picture was normal. Of the patients recovering, four had no polymorphonuclear cells whatever before treatment. Only one had a total count over 150 on admission. All had mouth lesions and some very severe ulcerations. In all patients who recovered, the first definite signs of clinical improvement occurred on the third or fourth day after treatment was begun, irrespective of the duration of the disease before treatment. General symptomatic improvement and healing of the ulcers occurred simultaneously with the hematologic improvement. In cases reported in the literature, with other types of treat-
ment, improvement almost always took place after a much longer interval and the temperature remained high for a much longer time. The charts of temperature and blood cell counts were practically identical in all cases - not only of granulocytopenia, but also of patients with malignant neutropenia associated with infection. Their mortality in this first series was 33 per cent in adequately treated cases, but a more recent report, over a larger series of cases, shows a mortality of 26 per cent. The first sign of improvement usually occurred between the third and seventh day, and was usually on the fifth day. The total and differential counts were invariably normal in ten days, sometimes in eight. They comment: "The consistency with which the reaction occurred on or about the fifth day is of great significance. It is at this time that the reticulocyte rise begins to take place following liver therapy in cases of pernicious anemia." This reaction would only tend to show that the time for the maturation of granulocytes was about the same as that of the erythrocytes. We do not have any definite experimental proof, however, that the nucleotide K-96 supplies a maturation factor for granulocytes. The experimental work on nucleic acid and its decomposition products has shown only a chemotactic factor. Five of the cases in Jackson's first series, with no essential clinical differences from those of the recovered cases, died in spite of active nucleotide therapy. This brings up the possible difference between the pathology of benign neutropenia and malignant neutropenia, i. e., the function of granulopoiesis was depressed, maturation had ceased, and the bone marrow was aplastic in the fatal cases; while in benign cases the function of granulopoiesis was arrested, maturation had not ceased, and the bone marrow was not exhausted but was
normal or hyperplastic, the chief fault seeming to have been in the delivery, or lack of a chemotactic factor. It is only reasonable to assume that if the cells are not developing they cannot be called into the circulation.

The acute cases in Jackson's series were given 0.7 grams of nucleotide K-96 in 100cc. of saline solution intravenously, daily for four days, and 0.7 grams in 10cc. of distilled water, given in addition, intramuscularly, on the same days and on each subsequent day until there was definite improvement. The intramuscular injections usually give no reaction; the intravenous injections give a sharp, temporary reaction sometimes.

MISCELLANEOUS AGENTS — Any agent that is thought to be capable of stimulating the bone marrow to production of granular cells may be worthy of a trial. Among these may be mentioned various non-specific protein substances, vaccines and sera of various types. None of these have proven to be of any definite value. In the past it was thought that an agent introduced subcutaneously which would cause a local abscess would in that way stimulate the production of neutrophilic granulocytes.

Turpentine was one of the substances used for this purpose. Such agents are rarely used now, as they have not been shown to have any therapeutic value. Intravenous injections of gentian violet and acriflavine have been given, but they will not stimulate the production of granulocytes. They were perhaps used with the idea of a blood stream infection in mind. The injection intravenously of dead typhoid bacilli has been used, but this is a dangerous procedure in as much as neutropenia has been known to follow typhoid prophylaxis. (33) Fetal liver and extract of bone marrow have been administered, but no definite results were obtained. One author used massive doses
of liver extract by mouth with recovery of the patient, in spite of the fact that the white cell count was below 1000 with complete absence of the neutrophils. Since the stimulating effect of this substance of the erythroblastic function of the bone marrow is well known, there may be some rational grounds for this form of therapy. (6) Squibb's leukocyte extract, in daily intramuscular injections of 50cc., divided into two doses, has been used in many cases along with transfusions and other forms of therapy. Definite results were lacking and they are seldom used now. The recently marketed preparations of colloidal sulphur have been shown to increase the leukocyte count from normal to as high as 30,000, in syphilitics, and may be worthy of a trial.

TREATMENT OF LOCAL LESIONS - The second object in the treatment of granulocytopenia is the control of the local infection. As pointed out above, this disease is characterized by an early breaking down of the normal protective mechanism afforded by the neutrophilic granulocytes. With these granulocytes absent, the organisms ever present in and on the body quickly invade. Therefore, infection, local or general, is the most frequent complication of granulocytopenia. The most frequent local lesions are in the mouth and throat. An antiseptic that will not cause a chemical destruction of the tissue is advised. For local treatment to the oropharyngeal lesions Hamburger (30) advises the following:"after nourishment the mouth and throat are sprayed with a saturated solution of potassium chlorate, a compressed air atomizer being used. Following the spraying, each ulcerated area and the gums are swabbed with a solution of copper sulphate, ten grains to the ounce. This oral treatment is given as often as five times a day, less as improvement
takes place.

Phlegmonous masses in any locality should not be incised unless absolutely necessary, since no local abscess forms under the existing granulopenic state. After the neutrophilic granulocytes return to the blood stream in sufficient numbers, abscesses may form, and incision and drainage may be instituted, if necessary. However, when the neutrophilic granulocytes return many pathological conditions will right themselves. Surgical procedures of any type should not be attempted in these patients, as it is dangerous in the presence of a leukopenia, especially a neutropenia. Such a procedure may bring about a sloughing of tissue, as healing cannot take place normally in the presence of a leukopenia and neutropenia. There are cases on record in which necrotizing changes have appeared in the skin at the site of venipuncture. (1)

It is thought by some authors that whole blood transfusions may be of some value in preventing the entrance of infecting organisms into the blood stream. It is important to keep the oral and nasal membranes as free of organisms as possible, particularly in the chronic and recurrent cases. Every effort must be made to protect the patient from exposure to infections, particularly of the upper respiratory type. The food and fluid intake during the acute stage should be maintained at as high a level as the clinical condition will permit. The removal of foci of infection must only be done with the greatest of care.
SUMMARY

1. Granulocytopenia is probably a true disease entity with definite symptoms and clinical course. It may, for the sake of convenience, be divided into primary (etiology unknown) and secondary (etiology known).

2. The disease is shown to have three onsets: a bone marrow onset, a blood stream onset and a clinical onset.

3. A specific and satisfactory treatment for cases having aplastic myeloid tissue has not yet been discovered; recovery if it occurs is probably spontaneous.

4. In those cases with normal or hyperplastic myeloid tissue, the nucleotide K-96 probably gives the best results.

5. Every throat infection which seems unusual in its manifestations and every rectal lesion which has an unusual appearance should indicate a white blood cell count without delay, for it is only by the early recognition and prompt treatment that one can hope to deal successfully with the disease when it does occur.
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