

5-1-1940

## Granulocytopenia

John P. Heinke  
*University of Nebraska Medical Center*

This manuscript is historical in nature and may not reflect current medical research and practice. Search [PubMed](#) for current research.

Follow this and additional works at: <https://digitalcommons.unmc.edu/mdtheses>

 Part of the [Medical Education Commons](#)

---

### Recommended Citation

Heinke, John P., "Granulocytopenia" (1940). *MD Theses*. 808.  
<https://digitalcommons.unmc.edu/mdtheses/808>

This Thesis is brought to you for free and open access by the Special Collections at DigitalCommons@UNMC. It has been accepted for inclusion in MD Theses by an authorized administrator of DigitalCommons@UNMC. For more information, please contact [digitalcommons@unmc.edu](mailto:digitalcommons@unmc.edu).

GRANULOCYTOPENIA

-BY-

JOHN PAUL HEINKE

SENIOR THESIS

-1940-

PRESENTED TO THE  
UNIVERSITY OF NEBRASKA  
COLLEGE OF MEDICINE

481128

## GRANULOCYTOPENIA

Since the advent of this so-called disease entity some eighteen years ago there has been little mention of it in the text-books of medicine, many authors even refusing to grant the existence of such a process. Most mention is made of this disease in the journals where hundreds of cases have been cited, many of these being doubtful, the disease being merely a dumping ground for conditions showing pharyngeal lesions and having an unusual blood picture. The past few years have shown a marked increase in the number of cases, this being due, perhaps, to the profession becoming aware of the condition. After reviewing several hundred cases, it is my impression that this increase parallels the rise of chemotherapeutics where drugs are either given continuously over a long period of time or where a certain concentration of the drug within the blood is desired.

### DEFINITION

Granulocytopenia is an acute medical emergency of doubtful etiology characterized by a marked leukopenia with a drastic reduction in the percentage of granulocytes and by lesions of the gastro-intestinal tract, the latter usually being situated in the mouth or pharynx. The disease may be acute or chronic, the majority of the reported cases being of the acute variety.

### NOMENCLATURE

The credit for discovering and naming this disease is given to Werner Schultz<sup>1</sup> who in 1922 gave the name of "Agranulocytosis"

to a syndrome characterized by leukopenia, loss of granulocytes, pharyngeal lesions, and jaundice. As stated by Beck<sup>2</sup> this term is obviously incorrect since the word "agranulocyte", was originally intended for a condition in which the neutrophils lacked granulations. "Agranulocytosis" would therefore mean an increase in the number of neutrophils lacking granulations which condition is not intended. In 1923 Friedmann<sup>3</sup> gave this syndrome the term "Agranulocytic Angina" since the throat lesions, in his opinion, were the most important manifestations and the blood picture of secondary importance. This term is also unfortunate since the pharyngeal lesions are only a manifestation of the disease due to the loss of granulocytes and the protection they give mucosal surfaces which are at all times grossly contaminated. (Baldrige and Needles<sup>4</sup>). Due to the inability of either of these terms to clarify the disease picture numerous attempts were made to find a name which was both descriptive and accurate. The result has been a confusing array of terms all applied to the same disease entity. Baldrige and Needles<sup>4</sup> did not feel that the present knowledge was adequate to divide the syndrome arbitrarily and suggested that all cases of unexplained neutropenia with secondary infection be grouped under the general head of idiopathic neutropenia. Roberts and Kracke<sup>5</sup> gave the disease the name "agranulosis" since it indicated the pathological process as clearly and possessed the added virtues of brevity, simplicity,

and easy pronunciability, the latter being no small factor in my estimation. Fitz-Hugh and Krumbhaar<sup>6</sup> suggested the name "pernicious leukopenia", inasmuch as there is an absolute reduction in lymphocytes in the blood stream as well as neutrophils and because of certain analogies with pernicious anemia. Lamb<sup>7</sup> suggests referring to the idiopathic type of the disease as the Schultz syndrome of pernicious neutropenia just as one refers to the Addison-Bierner type of pernicious anemia. Rose and Houser<sup>8</sup> regard the use of the terms "agranulocytosis" and "agranulocytic angina" as unfortunate and suggest the use of a descriptive term such as "sepsis with granulocytopenia", or "agranulocytic infection", as suggested by David<sup>9</sup> and Feer<sup>10</sup>. The theory behind the two preceding terms is that the leukopenic state is due to an overwhelming infection, the basis for which has not been borne out by later experimental work and reports of cases. Little can be gained by discussing the brain-children of various authors who are at odds regarding the etiology and significance of the disease process. In this paper the disease will be referred to as granulocytopenia, this term covering all cases characterized by the loss of granulocytes.

#### HISTORY

The credit for recognizing the syndrome is given to Schultz<sup>1</sup>, who, in 1922, described what he believed to be a disease entity characterized by the following: (1) sudden onset with high fever and general malaise. (2) ulcerations, necroses, diphtheritic or gangrenous processes, especially of the tonsils,

pillars of the fauces, uvula, palate and pharynx and occasionally similar lesions of the gums, tongue, larynx, and genitals.

(3) absence of hemorrhagic diathesis. (4) presence of icterus. (5) Occasional enlargement of the liver and spleen. (6) characteristic blood picture--profound depression of the white blood cells, usually less than 1000, with disappearance of the polymorphonuclear leukocytes, the red blood cells and platelets not being disturbed. (7) rapid fatal course. (8) almost complete absence of granulocytic cells in the bone marrow. The spectacular nature of the disease makes one wonder under what name it was classified prior to 1922, since it seems highly improbable that such a condition did not exist before that time, although it is Beck's<sup>2</sup> opinion that had it existed before it would have been described. Although no definite conclusions can be drawn, it is interesting to note that in 1870, before the era of blood counts, Peter,<sup>11</sup> in writing of gangrenous angina, describes the gross characteristics of the blood quite carefully. Mackenzie<sup>12</sup>, in his "Manual of Diseases of Throat and Nose", lists a condition under the heading of "Putrid Sore Throat" and defines it as follows: "Primitive gangrene of the pharyngeal mucous membrane, constituting an affection per se, and originating independently of any other malady, such as diphtheria, scarlet fever, etc." Trousseau<sup>13</sup>, in 1873, mentions four cases, two of which definitely answer the description of agranulocytic angina. He also cites a case of Gublers<sup>14</sup>, in which a malignant membranous and gangrenous sore throat, complicated by diphtheria of the nasal fossae was present. It is my impression, therefore, that the syndrome was not new since

together with the foregoing descriptions, Brown<sup>15</sup>, in 1902, reported a fatal case of acute primary infectious pharyngitis with extreme leukopenia, Turk<sup>16</sup>, in 1907 a case of severe sepsis with a low granulocyte count, and Leale<sup>17</sup>, in 1910, reported a case in a male child, under the title, "Recurrent Furunculosis in an Infant Showing an Unusual Blood Picture". This latter case is interesting in that twenty years later the patient, then twenty-one years old, was reported as a case of "Recurrent Agranulocytosis" by Rutledge et. al.<sup>18</sup>. The case reported by Lovett<sup>19</sup>, in 1924, was the first one recognized in America and Lauter<sup>20</sup>, in 1924, was the first to report a recovery from the disease. Since 1922 several hundred cases have been reported with the number of annual cases increasing.

#### PHYSIOLOGY

Since we are dealing with a problem that involves the leukocytes it is only fitting that something be said of their formation. From the light of present day knowledge it is the consensus of opinion that the disease process is due to an injury of the leukopoietic tissues, the details of which will be dealt with later. As stated by Beck<sup>2</sup> "On the basis of this knowledge of the physiology of granulopoiesis, a theory of pathogenesis may be formulated".

The leukopoietic or more specifically the granulopoietic tissue is located in the red marrow, found in the ribs, the vertebrae, the sternum, the bones of the skull, and the



os innominatum. The sum of the bone marrow, as determined by Toppich<sup>21</sup>, in two newly born infants, was equivalent to approximately 2.3 per cent of the body weight. Since infant bone marrow is practically all of the red type, this figure represents the functioning mass. Mechanik<sup>22</sup> determined the marrow volume of thirteen adult cadavers and found the marrow mass to vary from 1600 to 3700 gm. with an average of 2600 gm. He believed the red and yellow portions of the marrow were about equal, giving an average value of about 1300 gm. to the active portion, equivalent to approximately 2.3 per cent of the body weight. Wetzel<sup>23</sup> reported a red marrow volume of 1419 c.c. in the skeleton of a twenty year old man. This would compare favorably with that reported by Mechanik<sup>22</sup>. Nye<sup>24</sup> in experimental work upon the bone marrow volume of rabbits found the number to be 2.6 and 2.0 per cent in two rabbits examined, making an average of 2.3 per cent. This figure is identical with the figure found for new born infants<sup>21</sup> and is one-half the value given for adults<sup>22</sup>. As can be readily seen, the granulopoietic system is an organ of no small size, being almost equal in size to the liver. The amount of granulopoietic tissue. (Doan and Zerfas)<sup>25</sup>. This seems odd when one thinks of the vastly greater number of erythrocytes than granulocytes but it must be remembered that the life of the red cells is some six times that of the granulocytes.

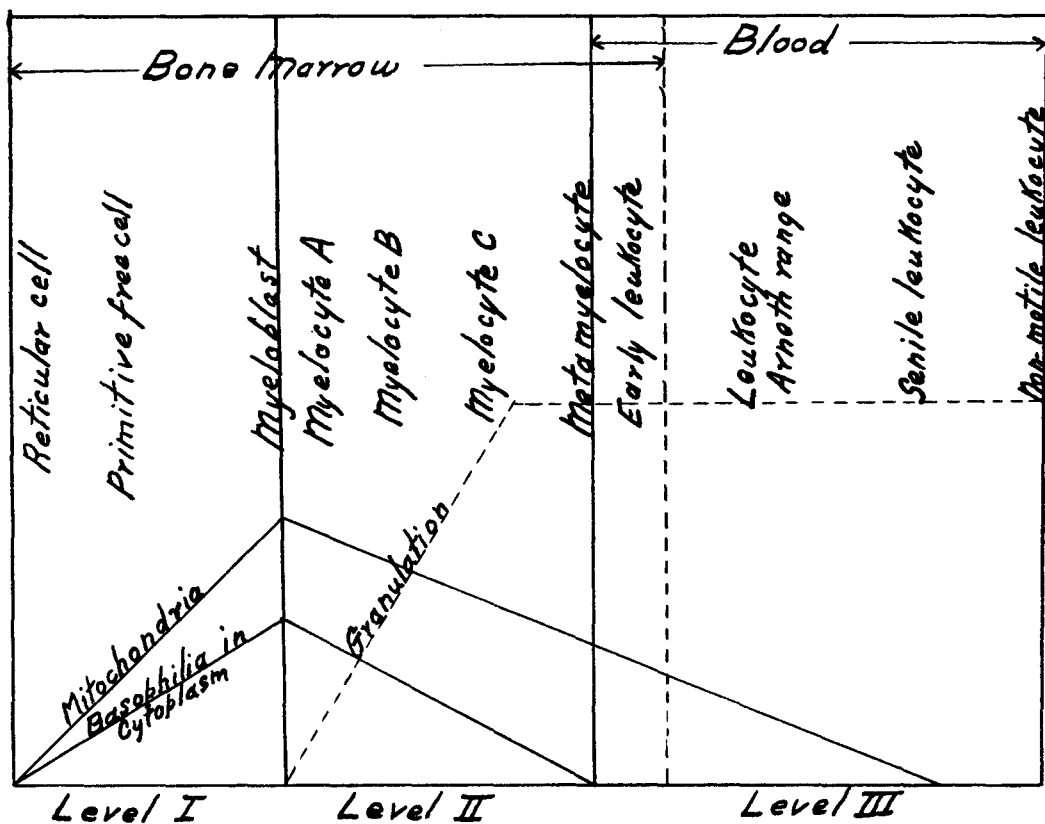
Doan<sup>26</sup>, in 1922, proved that the circulation of the bone marrow was closed, as in all organs of the body, but noted that the "transition capillaries" leading from arteries of the third and fourth order were not the functioning capillaries, but lead

to sinusoids which make the functioning vascular bed of the bone marrow. He found that this bed of venous sinuses were in far greater number than could be patent at any one time, due to the narrow confines of the marrow cavity. The closed sinuses or "intersinusoidal capillaries" were demonstrated to be the actual or potential site of red cell formation. (Minot<sup>27</sup>). Doan and Sabin<sup>28</sup> made the statement that the mechanism that controls the proportion of collapsed to dilated sinuses regulates, in part, the erythroid-myeloid ratio.

The granulocytic elements of the blood arise from reticular cells located outside the interspaces of the sinusoids. These cells grow toward the vessels with the most mature forms being nearest the endothelium of the vessel wall. As maturation proceeds the granulocytes mass up against the vessel, gradually wedging their way into the circulation, the endothelium closing behind them. (Doan, Cunningham, and Sabin<sup>28</sup>). It is interesting to note the place of formation of both erythrocytes and granulocytes and to correlate this with the oxygen supply. The granulocytic elements as they mature, move toward the source of the best oxygen supply indicating their need for a relatively high oxygen tension. The red cells, however, mature in a position of relatively low oxygen tension.

A discussion as to the origin of the granulocytes would entail a great deal of time and would leave the reader with no definite impression regarding the exact cell from which the granulo-

cytic elements arise. Many theories have been forwarded in regard to this question but in order to facilitate reading I will use the opinion of my choice and the one which strikes me as being the most logical, namely that of Sabin's<sup>29</sup>, which states that the granulocytes arise from a reticular cell lying in the interstices between the fat cells of the marrow. The accompanying chart is taken from Sabin's article and needs very little explanation.



In level one the reticular cell after repeated divisions gives rise to the primitive free cell. The basophilia of the cytoplasm increases, the numbers of the mitochondria of the free cell reach a maximum and the cell becomes a myeloblast.

By the processes of basophilia and mitochondria the myeloblasts develop into promyelocytes and carries them into level two.

Level two determines the type of cell which develop from the myeloblast. As the chemistry of the granules changes promyelocytes, eosinophilic, basophilic and neutrophilic myelocytes develop. In this level granulation increases as mitochondria and basophilia in the cytoplasm decrease. No cell division occurs in level two. In the latter part of this stage the cells gain ameboid movement and are known as metamyelocytes. These cells may appear in the blood stream.

The third level contains the granulocytes of the circulating blood which are of uniform size and content of specific granules. As these cells become older they lose their ameboid movement and become more fragile.

That the number of leukocytes in the circulating blood is variable was demonstrated by Sabin, Cunningham, Doan, and Kindvall<sup>30</sup>, who found a characteristic rhythm of the total white blood cells with an interval of approximately an hour's duration. The total number of leukocytes was found to be increased in the afternoon, regardless of whether food had been taken or not, and this entire increase was shown to be the result of an increase in the number of neutrophilic leukocytes. Shaw<sup>31</sup> showed the total leukocytes of man to exhibit two tides, each occupying approximately twelve hours. The day tide began in the forenoon, reached its height during the afternoon and

completed its ebb in the evening. The night tide started in the evening, attained its height in the hours after midnight and ebbed away in the early forenoon. The tidal waves were thought to be the summation of rapid oscillations in the number of leukocytes, the oscillations occurring at intervals of fifteen minutes. These variations occurring at fifteen minute intervals are probably due to the neutrophils dying out in showers, often in considerable numbers, and being promptly replaced. (Sabin et al<sup>30</sup>). According to Shaw<sup>31</sup>, the curve of the total neutrophils closely follows the course of the tides and is largely responsible for the characters of the latter, and his observations showed that the lymphocytes and other cells do not follow the tidal curve. Warner<sup>32</sup> in his work upon the diurnal leukocyte tide of the rat, found one curve with its low point at about 10 p. m., ascending at an increasing rate until 8 a. m. and then falling at a decreasing rate until the low point is again reached at 10 p. m. His work on the rat differed from Sabin's<sup>30</sup> and Shaw's<sup>31</sup> in that he found the lymphocytes more responsible for this diurnal tide than the polymorphonuclear leukocytes although all cell types share to a smaller extent.

Little is known of the factors concerned in maturation of the granulocytes. Bacon<sup>32</sup> and his co-workers considered that in infections the stimulus to an increased activity of the marrow comes from altered body proteins. Such bacteria

that produce a sustained leukocytosis introduce a maturation factor since they produce an increased division, growth and maturation of the less mature leukocytes in the marrow far beyond the normal amount. Since the use of liver extract in the treatment of pernicious anemia this substance has been used, more or less empirically, in the treatment of agranulocytosis, but to no avail. To sum up the knowledge of maturation factors of the granulocytic series it may be said that the stimulus given by infections is, at the present time, the only way by which the bone marrow will show any multiplication of young types of granulocytes.

More is known of the chemotactic factors of the bone marrow. Around the latter part of the nineteenth century it was known that the introduction of a nuclein solution would cause an increase in the number of granulocytes within five to ten minutes. (Ames and Huntley<sup>33</sup>) Vaughan and McClintock<sup>34</sup> isolated a nuclein from the blood and demonstrated its germicidal power. Wells<sup>35</sup> believed the key to the problem for leukocytosis and immunity was to be found in the principle of chemotropismus or chemotaxis. Tomlinson<sup>36</sup> also noticed an increase in leukocytes following the injection of nuclein solution. Doan, Cunningham, and Sabin<sup>28</sup> brought about a temporary leukocytosis by administering inactivated typhoid bacilli. This depleted the bone marrow of myelocytes and left only the promyelocytes and myeloblasts. Doan and his associates<sup>36</sup> showed

that there was an outpouring of new cells from the marrow within forty-five minutes to two and one-half hours after the injection of sodium nucleinate, adenine and guanine nucleotides. They also demonstrated the chemotactic effect of the nucleic acid with the massing of leukocytes around the patent sinusoids, a marked diapedesis into the vessels and vacant areas of marrow from which the granulocytes had been withdrawn. Doan<sup>37</sup> later gave the following conclusions regarding the use of nucleic acid and its degradation products:

(1) 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.

(2) Repeated large intravenous injections tend neither to exhaust nor to cause a malignant hyperplasia of the myeloid elements in normal animals.

(3) A short course of injections stimulates a myeloid hyperplasia of normal marrow without otherwise injurious consequences, which is reflected by a relative and absolute increase in the amphophilic granulocytes in the blood stream of rabbits.

That the cells of the granulocytic series are essential for the maintenance of life and health has long been recognized. Upon the recognition of the disease process "granulo-

cytopenia", this fact has been impressed upon the medical profession even more. Granulocytes are thought to be the source of complement and the amount of complement present in the plasma is probably due to the continued destruction of these cells. Metchnikoff<sup>38</sup> attributed the ability of the blood to destroy bacteria to the power of the polymorphonuclear leukocytes to devour bacteria or phagocytic power. Buchner<sup>39</sup> disproved this theory by freezing the cells and then thawing them. The power of the cells to destroy microorganisms was not diminished, proving the lethal action to be due to precipitins, bacteriolysins, or hemolysins.

Experimental work upon the normal life span of the polymorphonuclear leukocyte was done by Weiskotten<sup>40</sup>, who proved the existence of these cells to be from three to four days. The number of these cells necessary can be readily seen to be quite large, since they are motile for only two to three days and are intact for four days at the most. Following this they are excreted through the saliva and probably throughout the entire digestive tract. Although such an inference is premature in this part of the thesis it can be seen that when such a mechanism is removed from the body the mucous membranes are laid wide open to invasion by any bacteria present. With advancing years there is a reduction in the amount of granulopoietic tissue, the basic cells being replaced by fat and fibrous tissue.



### THEORIES OF PATHOGENESIS

The number of theories as to the etiology of granulocytopenia are as numerous as the number of terms applied to this condition. An attempt will be made to deal with these theories as they developed during the past eighteen years.

During the first eight years following the discovery of this process the majority of cases were found in women, most of these being past the age of forty. Hueper<sup>41</sup> found the disease to be 77.5 per cent in women but this was in 1928 before the disease had gained much headway as far as interest by the medical profession was concerned. Then too, Schultz had stated that the disease was predominant among women in that age group, which would tend to cause an error in diagnosis. Since that time it has been found that the disease is about equally distributed between men and women and although most of the cases are in adults it is by no means limited to this age group. The case reported by Leale<sup>17</sup> in 1910 was in an infant and showed the blood picture of granulocytopenia with recurrent furunculosis. Hueper and Garrison<sup>42</sup> mention the possibility of previous disease of the liver and gallbladder in cases of granulocytopenia. This has been shown by other authors but does not seem consistent enough to be of much importance.

The majority of the earlier authors regarded granulocytopenia as a direct result of overwhelming infection and some of them still maintain this view. (Fear<sup>10</sup> and Weiss<sup>43</sup>). Schultz<sup>1</sup> believed it to be an infectious disease of unknown origin with a special toxic affinity to the myeloid tissue. Other authors believed the disease to be a septicemia with an atypical reaction of the hematopoietic system either due to bacteria which possess a specific affinity and toxicity to the granulocytic system, (Zadek<sup>44</sup>, David<sup>9</sup>, Pelnar<sup>45</sup>) or due to an atrophy or low vitality of this organ in the presence of a virulent septic infection. (Zikowsky<sup>46</sup>). Fried and Dameshek<sup>47</sup> in their work upon the experimental agranulocytosis used *Salmonella suis* organisms intravenously in rabbits. A severe leukopenia developed rapidly and an almost complete granulocytopenia resulted. There was but little tendency toward regenerative activity on the part of the granulocytic series. The animals usually died within forty-eight hours after infection and the bone marrow, postmortem, usually showed but little evidence of regenerative activity. With smaller doses immature granulocytes appeared in the blood stream within three to four hours and at times a monocytosis developed, their number exceeding the number of lymphocytes. They concluded that there were close similarities between agranulocytosis in rabbits and that observed in man. Other authors found that a long-continued granulocytopenia could not be induced in healthy rabbits by the use of a small group of common bacteria. (Piersol

and Steinfield<sup>48</sup>). In regard to bacteria cultured from oral and pharyngeal lesions in cases showing the granulocytopenia blood picture there have been found Klebs Loeffler bacilli reported by Schultz<sup>1</sup>, Vincents spirochetes by Skiles<sup>49</sup>, Pietti<sup>50</sup>, David<sup>9</sup>, Bantz<sup>51</sup>, Moore and Wieder<sup>52</sup>, Pneumococci by Schultz<sup>1</sup>, Bantz<sup>51</sup>, Bacillus pyocyaneus by Lovett<sup>19</sup>. Blood cultures have shown Streptococcus viridans, (Cannon<sup>53</sup>), Streptococcus hemolyticus (Lanter<sup>20</sup>, Elkeles<sup>54</sup>), Staphylococcus by Schultz<sup>1</sup>, and Schultz and Jacobwitz<sup>55</sup>. It has been demonstrated numerous times that the granulocytopenia precedes the appearance of the mouth lesions. Three cases of granulocytopenia were seen to develop in the hospital while having routine blood counts. The patients were admitted for (1) unexplained jaundice (Ehrmann and Preuss<sup>56</sup>), (2) tuberculous arthritis (Bantz<sup>51</sup>) and (3) fracture of the tibia (Hunter<sup>57</sup>). An interesting case is reported by Roberts and Kracke<sup>58</sup>, in which the patient had recovered from the first attack. Before the period of the second attack the leukocytes were seen to diminish with the granulocytes bearing the brunt of the loss. Two days after this all polymorphonuclear leukocytes were absent but the patient was mentally alert and felt fine. On the morning of the third day the patient seemed to wilt before the eyes of the observers, areas of ulceration appeared upon the tongue and four days later the patient expired, having existed for seven days without a demonstrable granulocyte in the blood stream. The

predominate organism isolated from the sputum, throat, mouth ulcers, feces, urine and from embolic abscesses was Streptococcus hemolyticus.

From the foregoing dissertation it is quite evident that no particular organism is responsible for all cases, so the assumption may be drawn that the oral and pharyngeal lesions are the result of the loss of the circulating polymorphonuclear leukocytes which normally form a protective barrier for all mucous membranes, which are the least protected from the normal bacterial flora and are always contaminated. This view is concurred in by Doan<sup>59</sup>, Jackson<sup>60</sup>, Pepper<sup>61</sup>, Kastlin<sup>62</sup>, Baldrige and Needles<sup>4</sup>, and Roberts and Kracke<sup>5</sup>. As stated by Hueper<sup>41</sup>, "The demonstration of bacteria in the blood of patient's with granulocytopenia is not proof of the infectious nature of the disease, because bacteria are also frequently found in later stages of leukemias and their presence is due to invasion of the body through areas of necrosis."

The belief that granulocytopenia is the result of a septic or toxic action upon the myeloid tissue is held by a few men<sup>1, 46, 56</sup>. Stocke<sup>63</sup>, believed that granulocytopenia was not a specific entity but an abnormal reaction to sepsis, this belief being shared by Dameshek and Ingall<sup>64</sup>. As stated in the previous two paragraphs this conception is difficult to believe since in many of the cases the loss of granulocytes precedes the infection<sup>51, 56, 57, 58</sup>. Studies of the myeloid

tissue both before and after death in many cases showed a normal or increased number of myelocytes, this state causing Fitz-Hugh and Krumbhaar<sup>6</sup> to raise an objection to the hypothesis of "granulocytic aplasia" as constituting the "primary" pathologic mechanism of the disease and coined the term "maturation arrest" for consideration and future study. Rosenthal<sup>65</sup> had noticed this condition of the marrow in what he termed a benign form of granulocytopenia as the result of infection. Dennis<sup>66</sup>, after experimental work on rabbits in which they had produced granulocytopenia by embedding capsules of bacterial products in the tissues, concluded that pyogenic organisms, under conditions simulating a focal infection, are capable of producing a granulocytopenia, which may allow generalized infection and death. He suggested that granulocytopenia in man is due to the action of a leukocidin, rather than to a specific microorganism. It was his impression that pyogenic bacteria were capable of producing granulocytopenia only when they were restrained from active penetration into the tissues, yet they were so situated that their toxic products could be absorbed; otherwise they stimulated the leukopoietic system and produced a leukocytosis. Since the injection of filtrates of organisms had so consistently failed to induce a depression of the granulocytes (Kracke<sup>67</sup>), it appeared to Dennis that the constancy of the supply of toxin was a highly important factor in the production of a detectable leuk-

openia. For the most part these conditions are satisfied in a chronic focal infection.

The possibility of granulocytopenia having an allergic background in which the bone marrow is the point of least resistance has been mentioned to some extent in the literature but the nature of this action is not made clear, as there is some overlapping between sensitivity to organic compounds and to drugs and chemicals. Schilling<sup>68</sup> produced a blood picture similar to that of granulocytopenia by anaphylaxis and it is his impression that it may be an anaphylactic condition rather than an individual disease. Bromberg and Murphy<sup>69</sup>, and Kracke<sup>70</sup>, reported cases following the administration of prophylactic typhoid vaccination. Pepper<sup>61</sup> suggested that granulocytopenia might have allergy as the background, not of the angina but of the leukopenia, and cited cases to substantiate his theory. Kopelowitz<sup>71</sup>, believed that some factor, possibly allergy or endocrine, was present which rendered the granulopoietic system susceptible to a noxious agent. That some factor, whether inherent or acquired, which renders the bone marrow susceptible to the action of foreign proteins or to drugs and chemicals exists, is very probable, but to state that the condition is due to an allergic sensitivity I believe to be unverified.

There has been very little work done upon the nutritional aspect of granulocytopenia and little, if any, mention is made

of any dietary deficiency in any of the reported cases. In experimental work done upon dogs and monkeys with diets deficient in vitamin G (B<sub>2</sub>) it was found that together with the ulcerative stomatitis typical of the lack of this vitamin there was also a marked leukopenia and anemia. (Miller and Rhoads<sup>72</sup>, Day, Langston, and Shukers<sup>73</sup>). A maturation defect of the myeloid elements of the bone marrow was also found in the dogs which were given a diet deficient in the factor that prevents black tongue.<sup>72</sup>

Perhaps the most recent theory regarding the etiology of this disease is that in which the spleen is believed to be the causative factor. (Moore and Bierbaum,<sup>74</sup> Wiseman and Doan<sup>75</sup>). In three patients showing the granulocytopenic syndrome the spleens were removed and the white blood count returned to normal. Histologically each of the three spleens removed showed extreme clasmatocytosis with excessive phagocytosis of granulocytes. All other factors were ruled out in these three cases before splenectomy was performed.<sup>75</sup> The belief of Moore and Bierbaum<sup>74</sup> is that the neutropenia is produced by an abnormal phagocytosis of leukocytes by the spleen. Reisman<sup>76</sup> reported a case of chronic granulocytopenia which was treated by splenectomy in which the granulocytes returned to a normal level after failing to respond to pentonucleotide, liver extract, vitamins, and bone marrow concentrations. His belief was that the spleen had been exerting a hormonal de-

pressant effect upon the myeloid elements in the bone marrow.

The endocrine system as being an etiological factor was first mentioned by Friedmann<sup>3</sup> who believed in an endocrine disturbance which effects an impairment of the transportation of leukocytes from the place of production into the blood and also of the production itself of the myeloid tissue resulting in a decrease in the number of leukocytes in the blood. As he referred in his explanation mainly to the ovary as the gland involved, granulocytopenia in men would be excluded. Thompson<sup>77</sup>, and Jackson and Merrill<sup>78</sup>, have reported cases associated with the menstrual cycle. In seventeen out of eighteen young women with granulocytopenia, the onset of subjective symptoms occurred within a day or two of the onset of the regular menstrual period and all were menstruating upon admission to the hospital. One or more recurrences were observed in six of these eighteen patients and each recurrence preceded, by a day or two, the onset of catamenia.<sup>77</sup> He concluded that a relationship existed between the hormones associated with menstruation and the neutropenic episodes. Another case showing recurrences of the granulocytopenic syndrome was followed for seven months. All relapses came on the first day of the menstrual period following which the granulocytes would be absent for two to three days and then reappear but their rise was not up to the previous levels. With the use of pentonucleotide the white blood cells could be



raised prior to menstruation but the fall on the first day of menstruation could not be prevented. The conclusions reached in regard to the course taken by the leukocytes seemed to indicate that two factors were at work; one slowly progressive and fundamental, the other related to the menstrual cycle and periodically aggravating the first. Antuitarin-S was used prior to a menstrual period, after the granulocytes had been steadily falling for seven months, and 2 c.c. was given daily for ten days. There was a sharp rise in the number of total polymorphonuclear leukocytes on the first day of menstruation and they reached the highest level for seven months. A few days later the patient caught a cold, developed diarrhea, nausea and vomiting, and her white blood cells fell to a low level. Her condition became worse and the patient died on approximately the first day of her menstrual period<sup>78</sup>. Follow-up studies on a case of recurrent granulocytopenia previously reported,<sup>17,18</sup> was done by Thompson<sup>77</sup>. This patient, a male age 25, showed a granulopenia every twenty-one days. During the neutropenic period there was a drop in the amount of female sex hormone in the urine and at the same time there was an increase in Prolan. This man showed a great deal of female sex hormone in his urine and was sexually immature in regard to his genitalia, body-contour, etc. A cyclic decrease in the granulocytes had been noticed in this patient since he was an infant, the first report being prior to the discovery of the granulocytopenic syndrome.

Corey and Britton<sup>79</sup>, in reporting on the blood cellular changes in adrenal insufficiency, and the effects of cortico-adrenal extract, noted an increase in the red blood cells of 50 to 100 per cent following adrenalectomy. This was probably due to fluid loss but the total white blood cells were decreased to a similar extent, especially the neutrophils, with a relative increase in the number of lymphocytes. Upon the administration of cortico-adrenal extract to animals suffering from severe adrenal insufficiency and showing the above profound blood cellular disorganization, a complete restitution of the normal cell value was observed. Control experiments with adrenalin, glucose and saline solutions were negative, with the exception of the partly effective action of adrenalin on the white rat. They forwarded the possibility that the neutrophilopenia of adrenal insufficiency is related to the clinical condition of "agranulocytosis".

Hubble<sup>80</sup> postulated that bone marrow depression may be due to a pituitary insufficiency and concurs in the opinion held by others that granulocytopenia may be caused by cortical adrenal malfunction. Kunde, Gree and Burns<sup>81</sup> noted the production of a hypoplastic and aplastic bone marrow in thyroidectomized rabbits.

From the above evidence presented it seems very possible that granulocytopenia may have an endocrine malfunction as the basis for its etiology, with the primary injury, in cases where drugs have been taken, being on the endocrine system causing an imbalance with secondary injury

to the granulopoietic tissues due to the loss of this mechanism.

Reznikoff<sup>82</sup> presented a series of cases, in thirteen of which fatigue due to excessive work, lack of sleep and worry was more frequently encountered than any other etiologic condition.

Kracke and Garver<sup>83</sup> noticed leukopenia in patients exposed to excess sunlight. These patients when taken to higher altitudes recovered due probably to the fact that more clothes were worn and the amount of solar irradiation was decreased. This is the only reference made to leukopenia due to the sun's rays. Martland<sup>84</sup> made mention of the fact that internal radiation with x-rays can affect the marrow output in a number of ways, the occurrence of a simple leukopenia being rare, there being more often an affect upon the production of the red blood cells also.

According to the observations of Hoff<sup>85</sup>, there is some proof that the vegetative nervous system influences the circulating leukocytes. They mention the action of various drugs such as adrenalin, atropin, pilocarpin, and ingestion of milk as being examples of reactions on the vegetative nervous system. Mueller<sup>86</sup> showed that the intradermal injection of foreign protein, such as aolan, produces a peripheral leukopenia. He believed that there is a splanchno-visceral correlation, so that when the leukopenia occurred

in the individual blood vessels, there was a marked increase in the white blood cells in the splanchnic area. The injection of adrenalin produced a reversal of the leukocytic distribution. (Mueller and Hoelscher<sup>87</sup>).

The constitutional factor may be of great importance, and may explain the increased frequency of the idiopathic type of granulocytopenia in females. It is not infrequent for some of the patients who develop granulocytopenia to have a constant leukopenia or a tendency to leukopenia as a result of some primary disturbance of the granulopoietic tissues. These tissues may be somewhat diminished, or may be sluggish in production, or the bone marrow may become rapidly exhausted so that no defense mechanism exists. Whether certain patients have a constitutional and an abnormally functionally limited bone marrow for making blood is as yet a theoretical question.

The last and perhaps most important etiological agents rather than theories are drugs used in increasing amounts during the past few years. Plum<sup>88</sup>, Kracke and Parker<sup>89</sup>, hold that practically all neutropenic patients have taken drugs. Jackson<sup>90</sup> found that only 26 per cent of his patients up to 1934 had taken amidopyrine or allied drugs, 30 per cent had taken drugs but these could not account for the disease and 44 per cent had taken no drugs. Rosenthal<sup>91</sup> states that only 55 per cent of his patients developed granulocytopenia because of medication. The fact that granulocytopenia has increased

markedly in recent years, together with the known leukotoxic effect of the benzene, would seem to indicate that some, if not all, cases are due to the administration of drugs containing the benzene ring.

The first report of benzene being the cause of leukopenia was by Selling<sup>92</sup> in 1910 in which he noted cases of factory workers subject to benzol fumes. Two of these patients died and the bone marrow showed the white cells to be the most effected, especially the granular elements. Upon experimenting with pure benzene the same results were found as in the factory workers. (Selling<sup>93</sup>). Kracke<sup>93a</sup> later produced granulocytopenia experimentally by subcutaneous injections of benzene in olive oil and by intraperitoneal injection. The intravenous injection of benzene resulted in immediate death so the conclusion was drawn that the leukocyte-depressing properties of benzene were due to its oxidation products. Climenko<sup>94</sup> inhibited the leukogenic activity of the rabbit with certain cyclic compounds and upon postmortem examination noticed a marked degree of hyperplasia of the bone marrow with an increase in the number of primitive haemocyto blasts.

Kracke<sup>70</sup> was the first observer to incriminate drugs containing the benzene ring as an etiological factor in granulocytopenia. Amidopyrine was first stated to cause granulocytopenia by Madison and Squier<sup>95</sup> after they had produced a marked depletion of granulocytes by single doses of the drug

given to two patients. A few months later Benjamin and Biederman<sup>96</sup> reported a similar case produced by one dose of the drug. Kracke and Parker<sup>97</sup> collected 153 cases of granulocytopenia following the use of amidopyrine and concluded that in the clinical or experimental development of granulopenia it is necessary to presuppose the existence of a previously weakened, damaged or idiosyncratic bone marrow which may be congenital or acquired. Plum<sup>88</sup> collected 267 cases following amidopyrine.

The evidence incriminating this drug has been supported by clinical experiments in which patients who have recovered have been given small doses of the drug and the disease reproduced in them.<sup>88,95,96</sup> (Sturgis and Isaacs<sup>98</sup>). A clinical experiment by Dameshek and Colmes<sup>99</sup> with the intradermal injection of a mixture of blood serum and a solution of amidopyrine on four patients who had recovered from previous attacks of the disease resulted in strikingly positive reactions in three of the four and in two instances was followed by the development of the disease in all its clinical and hematological aspects. Skin tests by the scratch method were negative in these cases. They suggested a drug-protein linkage as the basis of the reaction. Herz<sup>100</sup> states that amidopyrine is a pyrazolon derivative containing the highly toxic N-H (imido) radical, is derived from Phenyl Hydrazine and resembles the latter in its rapid reduction of granular leukocytes in the blood stream and bone marrow.

Davis and Frissell<sup>101</sup> take issue with the statement that the benzene nucleus per se. or the benzene nucleus in assoc-

iation with a substituted amino group, is causative of granulocytopenia and the points against this reasoning are "(1) The benzene nucleus is the foundation stone for all aromatic compounds, hence, one must assume that all aromatic compounds are similarly toxic, which supposition we know to be false.

(2) If the benzene nucleus in association with an amino group is toxic, is it not curious that two of the most fundamental hormones in the human body, namely thyroxin and epinephrine, both contain the benzene ring in association with, although not attached to, an amino group. Incidentally, in that essential amino acid, tryptophane, the substituted amino group is attached directly to the benzene nucleus, and in many of the other essential amino acids the benzene nucleus is present.

It has been shown in Denmark<sup>88</sup> that prior to the advent of amidopyrine and its derivatives that the incidence of granulocytopenia was practically nil. As the use of the drug increased so did the incident of the disease until in 1934 the importation and use of the drug was prohibited following which the number of cases dropped to its previously low level.

It has been definitely proven, however, that some form of sensitivity must exist before amidopyrine will be toxic to the granulopoietic tissues since Rawls<sup>102</sup> used the drug in large amount on 400 arthritic patients. Of this number only four developed granulocytopenia, three of these dying of the disease. Hoff<sup>103</sup> cited three cases of the disease following the use of

allonal, an amidopyrine derivative, two of the patients dying, yet he had one patient who had taken two to four tablets every night for four years without demonstrable injury. Davis and Frissell<sup>101</sup> administered thirty grains of amidopyrine to thirty-two patients daily for from two weeks to three months with no change in the leukocyte count. It would seem, therefore, that the occasional person who acquires the disease after the administration of amidopyrine does so on the basis of a hypersensitivity to it and that the amount of the drug given plays little part in the production of the disease. In a report of the Council on Pharmacy and Chemistry of the A. M. A.<sup>104</sup> the conclusion was reached "that there is no question that amidopyrine is very important in the production of granulocytopenia", but no implication was made that all cases were due to the drug.

An interesting aspect of the disease concerning amidopyrine and its derivatives is that it appears most frequently in persons apt to be taking drugs, e. g. physicians, nurses, and those directly under the care of a physician.<sup>95</sup>

Krackel<sup>105</sup> prepared a list of drugs that should be used with caution due to the fact that they may depress the leukocytes. To this list may be added sulfapyridine, which is really a sulfanilamide derivative.

preparations containing aminopyrine

Allonal	Amidonine	Amifeine
Alphebin	Amidophen	Aminol
Amarbital	Amidopyrine	Am-Phen-Al
Amidol	Amidos	Ampydin
Amido-Neonal	Amidotol compound	Amytal compound



Analgia	Eu Med	Nod
Antabs	Gardan	Optalidon
Baramid	Gynalgos	Peralga
Barb-Amid	Hexin	Phenamidal
Benzedo compound	Ipral-Amidopyrine	Phen-Amidol
Cibalgin	Kalms	Phenopyrine
Cinchopyrine	Lumodrin	Pyramidon
Compral	Midol	Pyraminal
Cronal	Mylin	Seequit
Dymen	Neonal compound	Yeast-Vite
Dysco	Neurodyne	

drugs known to produce depression of the marrow

Dinitrophenol	Antipyrine
Novaldin	Sulfanilamide
Arsphenamine	Sedormid (thrombocytopenia)

In a similar manner to that of amidopyrine, dinitrophenol has been shown to be the apparent cause of the disease in certain instances. (Davidson and Shapiro<sup>106</sup>, Bohn<sup>107</sup>, Dameshek and Gargill<sup>108</sup>, Silver<sup>109</sup>, Imerman and Imerman<sup>110</sup>, Goldman and Haber<sup>111</sup>.) Nearly all of these patients were women who had taken the drug to reduce their weight. The drug had been taken in relatively large amounts and for the most part over a considerable length of time. The use of this drug attained the peak of its use rather rapidly and at the present time is condemned by the medical profession, there being much better methods of weight reduction without the untoward effects found after the use of dinitrophenol.

In addition to the previous mentioned drugs there are a few mentioned in the literature but are not of such great importance. Arsphenamine is perhaps the largest factor in this smaller group. Farley<sup>112</sup>, and Blew<sup>113</sup> reported cases following

the administration of arsphenamine, and Plum<sup>88</sup> reviewed the literature of the neutropenic diseases following the administration of arsphenamine with and without preparations of bismuth. In fourteen of these latter cases the hematologic defect was limited to the granulocytes. It was Farley's<sup>112</sup> impression that the direct cause was disintegration in vivo of the arsphenamines so that a benzol-like action takes place, the presumption being made that a preceding weakness of the hematopoietic system was present.

Other drugs being mentioned are Antipyrine (Groen and Goldman<sup>114</sup>), Acetanilid (Watkins<sup>115</sup>, and Hudnutt<sup>116</sup>), Phenacetin (Kracke and Parker<sup>117</sup>, and Costen<sup>118</sup>), Cinchophen (Shapiro and Lehman<sup>119</sup>), Pentavalent antimony compound (Neostibosan, Zia and Forkner<sup>120</sup>), Gold salts<sup>88</sup> (Taylor<sup>121</sup>), and Amytal (Watkins<sup>122</sup>). The barbiturates have been occasionally reported as etiological agents but in nearly all cases they were in combination with amidopyrine or one of its derivatives.

During the past three years a new series of drugs, or rather a drug and its derivative, have been used in large amounts by both the medical profession and the general public, the latter being due to the helpful hints given by medical columnists and unknowing reporters in search of something new to toss at a receptive public eager to try the magic medicine, unaware of its dangers or limitations. Sulfanilamide and its derivatives, which have proved to be of value in the treatment of hemolytic streptococcus infections and here of late in the treatment of staphylo-

coccic infections, is not without its dangers to the blood forming organs. Before any reports had been made of granulocytopenia caused by sulfanilamide, Fitz-Hugh<sup>123</sup> in 1937 stated; "Although I would not recommend these benzol-sulphonamid compounds promiscuously in cases with already established bone marrow suppression I think their theoretical danger is outweighed by their value in combating the hemolytic Streptococcus. Nonetheless, I predict that sooner or later this group of drugs may be added to the others which in a rare idiosyncrasy role may cause the syndrome of agranulocytic angina." It can be seen by this that medical men are recognizing in advance that drugs containing the benzene ring when given in large quantities will, in certain cases, cause a suppression of bone-marrow function.

Kracke<sup>105</sup> in reviewing the literature up to October of 1938 found eleven cases of granulocytopenia definitely due to administration of sulfanilamide. In every instance the patient had taken over 40 grams of the drug, which may indicate that the mechanism of granulopoietic depression is different from that following a single dose of amidopyrine. An observation made by Young<sup>124</sup> showed that serial leukocyte counts failed to give any warning of the impending injury to the granulopoietic tissue. A paradoxical case is presented by Ives<sup>125</sup> in which the patient had an idiopathic granulocytopenia with a complete loss of granulocytes and the typical ulcerations of the throat. This patient was treated with Prontosil and sulfanilamide for the streptococcus lesions of the throat and with pentonucleotide and liver extract

for the granulocytopenia. The patient recovered but there was a low number of neutrophils in the blood following recovery. The low neutrophil count was not significant since no conclusion could be reached as to whether it was due to the drug or to the disease.

Johnston<sup>126</sup> in remarking upon the value of premonitory signs and symptoms in regard to granulocytopenia following the use of sulfanilamide states that the most constant sign of impending disaster is pyrexia or an increase in pyrexia already present.

Experiments with cultures of human bone marrow and the effect of various concentrations of sulfanilamide were performed by Osgood,<sup>127</sup> who found that concentrations of less than 1-1000 did not grossly affect the growth characteristics of marrow cells of the majority of marrows for a period of eight days or less. Cultures containing 1-250 and 1-500 showed slightly lower counts on the fourth to the eighth day than the controls. This would indicate to me that the effect upon the granulocytes is not through the granulopoietic apparatus directly or either this drug is so changed by digestive and cellular processes that its products exert a toxic effect. This latter point might be a basis for an interesting experiment if a method could be evolved to subject the drug to processes occurring in the gastro-intestinal tract and the body tissues and then subjecting the bone marrow to the product obtained and noting the effect on the activity of that tissue.

In addition to the cases caused by sulfanilamide and its derivatives, Prontosil and Neoprontosil, there have been record-

ed, recently, instances of the disease caused by one of the newer derivatives, sulfapyridine. The toxicity of this drug seems to be less than that of sulfanilamide according to Llowy and Erskine<sup>128</sup>. The first cases following sulfapyridine were reported in England where the first use was made of the drug. (Johnston,<sup>129</sup> Coxon and Forbes<sup>130</sup>) Since then cases have been reported in the United States, (Dolgopol,<sup>131</sup> Rosenthal and Vogel,<sup>132</sup> Shecket and Price,<sup>133</sup> Shullenberger<sup>134</sup>) but in all probability the number of these cases will not be as great as those due to the parent drug, since the use of sulfapyridine has a more limited use, at least it has at present. The dosage of the drug needed in the treatment of pneumonia is considerably less than the amount of sulfanilamide needed for a streptococcic infection, (Barnett<sup>135</sup>) which together with the decreased toxicity of the former would tend to reduce the number of cases of granulocytopenia. In the future there will probably be cases of granulocytopenia recorded due to other sulfanilamide derivatives, sulfthiazol and sulfmethylthiazol.

#### . PATHOLOGY

The oral and pharyngeal lesions present in granulocytopenia differ from other inflammatory lesions in that no granulocytes are infiltrated into the surrounding tissues. Any cellular reaction occurring is due to the invasion of lymphocytes and macrophages. Although the mouth and throat are the usual sites of ulceration, the entire gastro-intestinal tract,

and vagina have been seen to show areas of necrosis. This is due to the fact that bacteria are present on their surfaces at all times and with the loss of the circulating granulocytes no protective mechanism remains, the bacteria simply invading a defenseless tissue. The lesion of the mucosa in the earliest stage is white with a yellowish cast, with a tendency to undermine to a spot  $\frac{1}{2}$  to 1 cm. away from the margin of the tonsil or gums.<sup>118</sup>

The pathology of the bone marrow is not agreed upon by numerous authors some of whom believe it to show an aplasia<sup>124, 58, 1</sup> (Jaffe<sup>136</sup>, Isaacs<sup>137</sup>), and others stating that the bone marrow shows a myeloid cell hyperplasia with a maturation arrest.<sup>6,4, 64</sup> After reviewing the literature it is my impression to liken this discussion to that of the blind men and the elephant, each of them having a different impression due to their having come in contact with different parts of the animal. So it is in this case where each author draws his impressions from the appearance of the bone marrow at the particular place from which it was taken. According to Jackson and Parker<sup>138</sup> confusion arises from too great a reliance being placed on the interpretation of stained smears taken from the bone marrow. He states that such smears are of importance in identifying certain cells not easily recognized in sections stained with eosin-methylene-blue, but no idea is given regarding the number or arrangement of the cells.

The impression of Schultz<sup>1</sup> was that the bone marrow

showed an aplasia and this was the impression under which the early authors on the subject laboured. Since that time the majority of reports have been to the contrary, the typical bone marrow lesion being a hyperplasia. 4, 6, 64, 101, 60, (Custer,<sup>139</sup> Schwartz, Garvin and Koletsly,<sup>140</sup>) Darling, Parker and Jackson <sup>141</sup> separate cases into two categories, the first being the rapidly fatal cases which show a lack of maturation in the granular series and hyperplasia of the stem cells, the second being cases where death took place after a longer period, these cases usually showing a hypoplasia of myeloid tissue with the coincident appearance of many plasma cells and lymphocytes. This is a logical way to attack the question since the time element should be of as much importance as in any other disease process. Another point that in my opinion has not received any consideration is that the cases showing myeloid hypoplasia were reported before the use of amidopyrine and allied drugs and were usually of the idiopathic type and probably of longer standing. The theory of "maturation arrest" <sup>6</sup> was proposed in 1932 after the use of amidopyrine had been in vogue for some time. These cases are for the most part rapidly fatal, the toxic product acting in greater concentrations over a shorter period of time.

Jackson and Parker <sup>138</sup> made the following generalizations after examining the marrow from twenty-five patients who died in various stages of what seemed clinically and hematologically to be classical agranulocytosis. "The marrow of the vertebrae,

ribs, sternum, and mid femur were essentially the same. The degree of cellularity was usually normal. Rarely the femur remained fatty, as in the normal adult. It would seem that in these instances death occurred too soon for the marrow activity to spread peripherally, although this view cannot be advanced with any enthusiasm. In some sternal and vertebral marrows, particularly in patients dying later in the disease, a certain amount of hypoplasia existed. On the other hand in the fulminating cases the marrow was perhaps unusually rich in cells. The degree of cellularity, however, varied but little and is, we believe, an unessential feature of the condition." Later in their article they make the statement that in those patients who survived the ravages of infection for a considerable period (eight to twenty days) the bone marrow picture was somewhat different. It was then found to be relatively hypoplastic.

There is little, if any, disturbance of the red cell formation. Erythroblasts, normoblasts, and nucleated red cells occur in normal numbers and show no abnormal features. <sup>138</sup> Megakaryocytes and platelets are also in normal numbers and of normal character. <sup>136, 138</sup>

As regards the pathology of other organs of the body it may be stated that most of these are secondary and due to septicemia following the loss of the granulocytes and the invasion of bacteria into the blood stream. In the three cases reported



by Wiseman and Doan <sup>75</sup> the spleen when removed during life showed extreme clasmatocytosis with excessive phagocytosis of granulocytes. This is the only report of such a finding.

#### LABORATORY DATA

The use of the laboratory data is very important in this disease since in many cases the differential diagnosis can only be made through the use of continued total and differential counts.

The characteristic features of the disease demonstrated by the use of blood counts are the marked leukopenia and granulopenia. The total count in nearly all cases of any severity is below 2,000 and is usually below 1000, at times falling to as low as 200 to 600. Even more typical than the low white count is the even lower granulocyte count, in the majority of cases these cells being completely absent or at most 2 to 3 per cent. The few cells that do remain are usually of normal morphology <sup>2</sup>. Beck <sup>2</sup>, in 1933 stated that in most of the fatal cases of this disease, the maturation factor is absent, but the chemotactic factor is present, causing the granulocytes to continue being delivered to the circulation until the supply is exhausted. But as has been stated previously most cases show a plentiful supply of immature granulocytes in the marrow, so in these cases it is probable that the chemotactic factor rather than the maturation factor is absent. If such is the case then the nucleic acid derivatives should

be effective in the treatment and are to a limited extent, but not as much as would be expected.

The viability of the circulating granulocytes during an attack of the disease has been reported upon by only one author<sup>18</sup> who states that preceding the onset of the attacks of cyclic granulocytopenia the life of the cells was about one-half of the normal time, the motility being reduced also.

Usually the lymphocytes are at first present in about their normal numbers, but later in the course of the disease there is an absolute decrease. No reason can be given for this reduction.

The erythrocytes, in a true case of granulocytopenia, are usually in normal numbers although later in the disease when there has been blood stream invasion by bacteria the red count may fall due to these secondary factors.

In idiopathic cases of the disease the blood platelets are normal in amount and formation but in cases due to drugs, more especially to benzene, there is a marked reduction.

There is insufficient proof of the toxicity of the serum in this disease. It has been reported that when leukocytes of a normal person were mixed with the serum from a patient with granulocytopenia, the motility was lost and some of the cells became dissolved.<sup>18</sup> This finding was not borne out in another experiment where the whole blood from two patients, one normal and one with the disease, was oxalated and mixed. No neutrophilic

destruction could be shown by this experiment but the motility or viability could not be determined.

Blood cultures are in reality of very little importance as far as any knowledge into the disease is concerned since they are merely secondary invaders having no direct relationship to the disease process.

The bacteria that have been removed from the throat and cultured have been reviewed in another part of this paper and are also of little significance since all of them except the Vincent's organism are found normally in that location.

#### SYMPTOMS

In the earliest stage of the disease the symptoms are due to the loss of the polymorphonuclear leukocytes. Usually by the time the patient has become ill enough to send for a physician he has already undergone the first three stages of the disease mentioned by Roberts and Kracke<sup>5</sup>. These stages are: (1) Bone marrow onset, (2) Blood stream onset, (3) Clinical onset. Prior to the time when the physician sees the patient the complaints are usually fatigue, weakness, frequent headache, or fever of the occult type, all of the symptoms being out of proportion to the physical findings. Closely following the above symptoms is the complaint of sore throat which may be scratchy in character. This is soon followed by ulcerations of the mouth and pharynx, together with a fever and chill. The breath is usually of a foul odor and the tongue is often heavily coated. In one reported

case where routine blood counts had been done and the granulocytes seen to disappear before the clinical onset the patient seemed to wilt and become so weak that she could hardly sit up. <sup>58</sup> The severity of the symptoms is largely dependent upon the degree of diminution of granulocytes with collapse occurring in the most severe type of granulocytopenia. Following these symptoms delirium and coma usually supervene and the patient dies unless the therapeutic measures are effective.

The temperature ranges from 101 to 106° and is the continuous type.

As far as previous health is concerned a great many of the patients give a history of tiredness or weakness. The question is raised as to how much of the weakness in acute infectious diseases is due to the disease itself and how much to the granulopenia and whether or not an increase in granulocytes stimulates strength and energy.<sup>5</sup> The complaints of weakness, exhaustion, or fatigue are twice as frequent in the granulopenic patients as in those with normal white counts.<sup>5</sup>

The granulocytopenic state may be chronic or acute, subacute, recurrent or cyclic. The chronic condition of moderate leukopenia may either mean a normal physiologic equilibrium maintained at a level somewhat lower than the average with no detectable influence on the normal health of the individual, or it may reflect a low myelocytic reserve in the marrow with constant potential danger of marrow insufficiency and decompen-

sation 37. The acute fulminating type presents a crisis that is rapidly fatal if recovery of marrow function is not initiated promptly. The subacute type shows a more prolonged illness characterized by 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. The recurrent type of granulocytopenia may occur weeks or months apart 70. The symptoms when the disease is in progress are usually similar to those of the acute or subacute type. The patient may die during one of these attacks or may completely recover. The cyclic type shows a characteristic regularity in which the attack can be forecast almost to the day. 17, 18 (Embleton 143) Some of these cases are associated with the menstrual cycle, the fall in granulocytes preceding the onset of menstruation, 77, 78.

#### DIAGNOSIS

The diagnosis of this disease is dependent upon the demonstration of the granulocytopenic blood picture, the lethargy, weakness, malaise, headache and the throat ulcerations. But to wait for the throat ulcerations will mean that many patients will be lost whereas they might have recovered, had the diagnosis been made sooner. The patients are usually sick for days, weeks or even months before the typical picture of granulocytopenia presents itself, during which time treatment can be instituted and prognosis bettered. The answer to this problem of diagnosis is more frequent blood counts upon those patients with ill defined

complaints where no physical signs are present. The history is of equal importance due to the possibility of the patients having taken some of the drugs known definitely to be etiologic in this condition. The incidence of those cases which have taken drugs has been mentioned elsewhere in this paper. The use of sternal marrow biopsies has been stated to be of use in diagnosing the condition,<sup>2</sup> but since the marrow varies so much from one part of the body to another it would seem that the only conclusion that could be reached by this means is whether the marrow is aplastic, hyperplastic or normal, according to the region from which it was taken. This one biopsy would fail to indicate the condition of the marrow elsewhere in the body.

#### DIFFERENTIAL DIAGNOSIS

In differentiating the throat lesions of granulocytopenia from those of Vincent's angina, diphtheria and acute follicular tonsillitis, a routine leukocyte and differential count is necessary together with smears and cultures from the ulcerated area. In writing on the differential diagnosis between diphtheria and oral lesions of blood dyscrasias Gordon and Litvak<sup>144</sup> state that a complete blood study is indicated in every case of suspected diphtheria in the presence of any of the following:

- (1) Persistence of the membrane after the administration of diphtheria antitoxin.
- (2) Lesions in the mouth in addition to those on the tonsil.

- (3) Cutaneous and mucous membrane hemorrhages.
- (4) Marked pallor.
- (5) General glandular enlargement.
- (6) Enlargement of the spleen.

In other diseases of an acute nature that show a leukemia the white count rarely goes below 3,000 and granulocytes are usually elevated.

The aleukemic type of acute leukemia or acute leukemia in the aleukemic phase very closely simulates granulocytopenia and the two diseases are frequently mistaken for one another. The findings which would point toward a diagnosis of leukemia are:

- (1) Absence or marked diminution of platelets.
- (2) Anemia of moment, particularly if progressive.
- (3) Hemorrhages, especially from the mucous membranes of the mouth.
- (4) Notable enlargement of the spleen and enlargement of lymph nodes, not readily explainable by adjacent ulceration and infection.<sup>141</sup>

In the majority of leukemia cases the blood smear shows a great number of immature cells, while in granulocytopenia it is rare for young forms to make their appearance.

The aplastic anemias must be differentiated in making a diagnosis of granulocytopenia. In the former there are subcutaneous hemorrhages and hemorrhages from the mucous membranes. The bone marrow is for the most part aplastic and is completely barren of erythrocytes, leukocytes and megakaryocytes which is different from that of granulocytopenia in which if the marrow is aplastic the erythropoietic and megakaryocytic function is not disturbed.

Other diseases which must be considered in making a differential diagnosis are infectious mononucleosis, pernicious anemia in the aplastic stage, and metastatic bone involvement. In infectious mononucleosis the injection of foreign protein such as aolan will cause an increase in the peripheral leukocytes and in this disease the onset is not so abrupt and severe as in granulocytopenia.

#### TREATMENT

The treatment of this disease as a whole is quite unsatisfactory. During the first few years following its discovery the majority of attention was paid to the oral and pharyngeal lesions, but since the medical profession has come to the realization that these are secondary, the main part of the treatment is directed toward stimulating the bone marrow to function.

The first essential in the treatment of granulocytopenia is that any drugs that the patient has been taking should be stopped immediately, since from 40-60% of the cases are due to the ingestion of amidopyrine or the newer sulfanilamide and its derivatives.

Blood transfusion is used in nearly every case and perhaps it is beneficial in a few cases. Theoretically and practically it is of very little use since the trouble is not due to an anemia, the red count usually being normal in all respects. Polycythemia has occurred due to excess erythrocytes following transfusion. (Griffith<sup>145</sup>) The number of white cells



given to the patient are relatively few in number and their duration in the blood stream is quite short.

In 1927 Friedmann<sup>146</sup> radiated the long bones in four cases giving one to three treatments at intervals of from two to several days. All four of the patients recovered. He suggested using 1/20 of an erythema dose over each of the bones. Later Friedmann<sup>147</sup> reported thirteen recoveries in fifteen nonfulminating cases uncomplicated by sepsis or pneumonia, treated exclusively by radiotherapy. In reality forty-three cases were treated but of these, twenty-three had either sepsis or pneumonia or died within thirty-six hours and all were discarded in so far as evaluating the effects of treatment was concerned. As stated by Jackson and Parker,<sup>141</sup> the mortality in the unexpurgated series was 32%. Call, Gray and Hodges<sup>148</sup> reported a case treated by irradiation that recovered. Taussig and Schnoebelin<sup>149</sup> treated four cases by irradiation with a mortality of 50%. I am not convinced that one of the cured cases was one of granulocytopenia. The two fatal cases treated by them showed a rise in the white blood count which was only temporary, the leukocytes falling in numbers soon afterward. Roentgen therapy had no effect upon its second administration. Gager and Speer<sup>150</sup> treated two cases successfully by irradiation, this being the only therapeutic agent employed. The rationale of employing a known bone marrow depressant in the face of an already depressed marrow is not sound reasoning. A temporary effect may be received by so-called stimulating doses of x-ray<sup>149</sup> but following

this the marrow can not respond to normal stimuli.

Splenectomy has been used in a few cases<sup>4,74,75,76</sup>.

but in most instances the patients condition is too poor to subject them to such a major procedure. Sufficient cases treated by this method have not been reported to warrant any statement as to its effectiveness.

Diet is no doubt a large factor in the treatment since it has been demonstrated that rations deficient in the vitamin B complex predispose to leukopenia and stomatitis.<sup>72,73</sup> A high vitamin diet would therefore be indicated, more or less empirically perhaps but of some value and certainly not harmful.

The use of transfusions of blood from patients who have recovered from the disease has been used (Markins<sup>151</sup>) with good results. This is not an indication of the effectiveness of the treatment since the patients had been given everything else prior to the transfusion of so-called immune blood with the recovery occurring at about the same time as spontaneous recovery would have come about.

The use of yellow bone marrow extracts has been used to some extent and good results reported by Marberg and Wiles<sup>152</sup> who treated twenty cases of granulocytopenia, obtaining a rise in the leukocyte count within twenty-four to thirty-six hours, this rise usually being to normal figures.

Parenteral administration of liver extract was first used by Foran<sup>153,154</sup> who reported good results by its intravenous

use in large quantities. Reich<sup>155</sup> in experimental work with liver extract upon rats noted the best response to occur in animals that had been subjected to benzol poisoning. He believed the liver extract probably acted by stimulating the bone marrow since the number of polymorphonuclear leukocytes were increased not only in the peripheral blood but in the bone marrow as well. He did make the statement that the results are far from startling so it is probablye that this method of treatment will be of little value in treating granulocytopenia.

The use of leukocytic cream has been used by Strumia<sup>156</sup> with good results in the treatment of severe neutropenias. This product is obtained by centrifuging the blood from several donors, after it has been treated to prevent coagulation. The layer of leukocytes is drawn off and injected intramuscularly. It is stated that following the use of this material there is a rise in the number of mature granulocytic cells in the circulation along with considerable clinical improvement. This response usually occurs within 48 hours following injection. The preparation of this material is rather time consuming and expensive due to the great amount of whole blood needed for the preparation of a few cubic centimeters of the leukocytic cream.

About the end of the last century a product known as nuclein was first used in the treatment of infection where the leukocytic response was inadequate. Vaughn and McClintock<sup>34</sup> isolated a nuclein in 1893 and demonstrated its germicidal power.

This substance was used with apparent success, an increase in leukocytes being noted within fifteen to thirty minutes following its injection.<sup>33,36</sup> Nuclein has been used very little in granulocytopenia but allied substances, adenine sulphate and pentonucleotide have been used to a great extent, the latter being the main treatment at the present time. Reznikoff<sup>157</sup> used adenine sulphate therapy in fifteen uncomplicated cases of granulocytopenia with recovery in eleven of the patients. He noted that in severely complicated cases of the disease or in aleukemic leukemia and aplastic anemia the drug was not effective in the doses used in the uncomplicated cases. The dosage used by him was 1 gram of adenine sulphate boiled in 35-40 cc. of saline and given intravenously t.i.d. for at least three days. This dosage was non-toxic. McLester and Parsons<sup>158</sup> treated two cases of granulocytopenia with adenine sulphate. One of these cases followed the use of typhoid vaccine and quinine and the other followed quinine alone. In the first case the response to treatment was an immediate, continued increase in white cells. The second case responded but more gradually over six weeks.

Doan, Zerfas, Warren and Ames<sup>159</sup> noted that the nucleotide products would raise the peripheral white count in normal animals and since then there has been much work upon this phase of treatment. In 1931 Jackson, Parker, Rinehart, and Taylor<sup>160</sup> treated twenty cases of malignant granulocytopenia with intramuscular and intravenous injections of nucleotides, fourteen of

the cases recovering. The time between administration of the drug and clinical and hematologic improvement was about five days. They believed that the nucleotides might have a definitely favourable effect on an inactive bone marrow in certain cases of malignant neutropenia. The term inactive is quite indefinite and leaves a doubtful impression as to just what condition of the marrow is inferred. In 1932 Jackson, Parker, and Rinehart<sup>161</sup> presented an analysis of 58 cases treated with pentose nucleotide K-96 with a mortality of 29.4%. The favourable clinical and hematological response took place rather sharply about the fifth day of the treatment, irrespective of how long the patient had been ill prior to the administration of pentonucleotide. Some of the cases were diagnosed as "malignant neutropenia", this term covering cases following a septic process, although they believed the prognosis to be the same as those of the idiopathic type for which they used the term "agranulocytic angina". 43 of the 58 cases were of the latter type, the mortality being 35% in this group.

The method of administration and dosages of pentose nucleotide are as follows:<sup>138</sup> "In the average case of agranulocytosis with a white count between 1000 and 2000 per c. mm., 10 cc. of Pentnucleotide are given intramuscularly two, or preferably, three times a day until the white count has definitely risen and young neutrophils have appeared. Ten cc. are then given once a day until the white count has been normal for several days. In cases which are extremely sick, and especially in those in whom

the total white count is below 1000, 40 cc. should be given per day until the white count has definitely risen and young neutrophils have appeared. The drug may be administered intravenously well diluted in saline by the continuous drip method, the speed of injection being such that no untoward reactions occur. If there has been no response at the end of ten days, further therapy with Pentnucleotide is useless."

Various other methods of therapy are the use of foreign protein, use of leukocytic extract, and the intravenous injections of gentian violet and acriflavine. None of these methods are preparations have been of any use in the treatment of this disease. It was also thought at one time and perhaps still is that the therapeutic introduction of bacteria subcutaneously in an attempt to cause abscess formation was beneficial in that the formation of granulocytes would be stimulated. This method of therapy was due to observations that recovery was noted following the spontaneous formation of abscesses. The rationale in this therapy is not sound, in that if infection and inflammation is needed there is plenty to be had in the throat ulcerations, without the introduction of more tissue injury.

The treatment of the throat lesions is of secondary importance and the main object is to relieve the pain, since no healing will occur until the level of the granulocytes is brought up to where the body will combat the infection. Warm saline throat irrigations are used and Hamburger<sup>162</sup> recommends spraying

of the mouth and throat with a saturated solution of potassium chlorate followed by swabbing of ulcerated areas and gums with a solution of copper sulphate, 10 grains per ounce.

Operations of election should be avoided in the face of the disease but in cases of empyema, ischio-rectal abscesses, and other instances of encapsulated and burrowing pus surgical intervention is indicated.

#### PROGNOSIS

The mortality rate in this disease has been decreasing since its discovery eighteen years ago. The disease was at first uniformly fatal due probably to a lack of knowledge as to its etiology, and to the fact that the oral and pharyngeal lesions were regarded as the most important factor and the treatment directed to this end. In 1927 Kastlin<sup>62</sup> reported a mortality of 95% and in 1931 Harkins<sup>151</sup> reviewed the literature, consisting of one hundred and fifty cases, the mortality being around 82%. Rosenthal<sup>65</sup> reported a series of twenty-six cases with a mortality of 46.2%. Taussig and Schnoebelen<sup>149</sup> reviewed three hundred and twenty-eight cases and found that the mortality was 75% without special therapy and miscellaneous forms of treatment. This series included the secondary types following drug therapy. In a series of 103 cases graduated according to the severity of the leukopenia it was found that among seven cases which had white counts of 2000 and over, the mortality was 16%, whereas 19% of

those whose white counts were 1000 to 2000 died and of those whose counts were below 1000, 55% died. It can be readily seen that prognosis becomes worse as the white count drops lower. The above cases were treated with Pentnucleotide, which is at the present time the best method of stimulating bone marrow, so those figures would be the most acceptable for the mortality rate at this time.

#### SUMMARY

(1) The term "granulocytopenia" is used to designate all cases of a disease characterized by the reduction in numbers of cells of the granulocytic series in the peripheral blood stream, secondary inflammatory lesions, usually in the mouth and pharynx, malaise, weakness and pyrexiz, irrespective of their course and duration, but exclusive of the diseases of a malignant nature and those affecting other elements of the hematopoietic system.

(2) The disease is probably on an allergic or hypersensitivity basis of an extrinsic or intrinsic nature.

(3) The pathology in the bone marrow, as to whether an aplasia or a hyperplasia with a maturation arrest is shown, is dependent upon the duration of the disease.

(4) The primary injury is possibly upon the endocrine system, causing a hormonal imbalance, with a secondary effect upon the bone marrow.



(5) Amidopyrine and sulfanilamide and its derivatives have been shown to have caused granulocytopenia, a preexistent sensitivity to the drug being assumed.

(6) The treatment of choice is at present Pentnucleotide.

(7) The mortality of treated cases is about 50%.

BIBLIOGRAPHY

1. Schultz, Werner: Gangraneszierende Prozesse und Defekt des Granulozeiten-systems, Deutsche med. Wchnschr. 48:1495, 1922 (From Beck, Ref. 2, and from Rosenthal, N.: Agranulocytosis, N. Y. State Hour. Med., 30: 695, 1930)
2. Beck, R. D.: Benign and Malignant Neutropenia, Arch. Int. Med., 52:239, 1933.
3. Friedemann, U.: Uber Angina Agranulocytica, Med. Klin., 19: 1357, 1923. (From Beck, Ref. 2).
4. Baldridge, C. W., and Needles, R. J.: Idiopathic Neutropenia, Amer. J. Med. Sci., 181: 533, 1931.
5. Roberts, S. R., and Kracke, R. P.: Agranulocytosis. Its Classification, Annals Int. Med., 5:40, 1931.
6. Fitz-Hugh, T. Jr., and Krumbhaar, E. B., Myeloid Cell Hyperplasia of the Bone Marrow in Agranulocytic Angina, Amer. Jour. Med. Sci., 183: 104, 1932.
7. Lamb, F. H.: Agranulocytosis, Jour. Iowa Med. Sc., 24:75, 1934.
8. Rose, E. and Hauser, E. K.: The Identity of so-called Agranulocytic Angina, Arch. Int. Med., 43: 533, 1929.
9. David, W.: Agranulocytosis, Med. Klin.. 20: 1614, 1924. (From Rose and Hauser, Ref. 8.)
10. Feer, W.: A contribution to the Question of Agranulocytosis, Schweiz. Med. Wchnschr., 56: 551, 1926. Abstr. Jour. A. M. A., 87: 336, 1926.
11. Peter: Dictionnaire Encyclopedique des Sciences Medicalés, Paris, Victor Mason et Fiels, 1870. (From Pepper, O. H. P.: The History of Agranulocytic Angina, J. A. M. A. 97:1100, 1931).
12. MacKenzie, M. A Manual of Diseases of the Throat and Nose, J. & A. Churchill, 1880.
13. Trousseau, A.: Gangrenous Sore Throat, Lectures on Clinical Medicine, 1: 323, 1873.
14. Gubler, A.: Memoir Sur L'Angine Maligne Gangreneuse, Archives Generales De Medicine, 9:513, 1857. (Quoted by Trousseau, Ref. 13).

15. Brown, P. K.: A Fatal Case of Acute Primary Infectious Pharyngitis with Extreme Leukopenia, *Amer. Med.*, 3: 649, 1902.
16. Turk, W.: Septische Erkrankungen Bei Verkummerung des Granulozytensystems, *Wien. Klin. Wochschr.*, 20:157, 1907.  
(From Editorial, *Agranulocytic Angina*, *J. A. M. A.*, 95:1428, 1930.)
17. Leale, M.: Recurrent Furunculosis in an Infant Showing an Unusual Blood Picture, *J. A. M. A.*, 54: 1854, 1910.
18. Rutledge, B. H. Hansen-Pruss, O. C., and Thayer, W. S.: Recurrent Agranulocytosis, *Bull. Johns Hopkins Hosp.*, 46: 369, 1930.
19. Lovett, B. R.: Agranulocytic Angina, *J. A. M. A.*, 83: 1498, 1924.
20. Lauter: Septic Tonsillitis with Agranulocytosis, *Medizinische Klin. Berlin*, 20: 1324, 1924.; (Abstr. *J. A. M. A.*, 83: 1466, 1924.)
21. Toppich, G., *Arch. f. Anat.*, 1914, 9. (From Nye, Ref. 24.)
22. Mechanik, N.: Untersuchungen über des Gewicht des Knochenmarkes des Menschen, *Zeitschr. f. Anat. U. Entwicklungsgesch.*, 79:58, 1926. (From Nye, Ref. 24.)
23. Wetzel, F.: *Anat. Anz.*, 53: 82, 1920-21. (From Nye, Ref. 24.)
24. Nye, R. N.: Bone Marrow Volume in Rabbits, *Soc. for Exper. Biol. and Med.*, 29:34, 1931.
25. Doan, C. A., and Zerfas, L. G.: The Rhythmic Range of the White Blood Cells in Human Pathological Leukopenia and Leukocytic States, with a Study of Thirty-two Human Bone Marrows, *Jour. Exper. Med.*, 46: 511, 1927.
26. Doan, C. A.: The Circulation of the Bone Marrow: Contributions to Embryology, *Carnegie Publications*, 14:27, 1922.
27. Minto, G. R. *Proc. Boston Nat. Hist.*, 29: 185, 1901.
28. Doan, C. A., Cunningham, R. A., and Sabin, F. R.: Experimental Observations upon the Origin and Maturation of Avian and Mammalian Red Blood Cells, *Contributions to Embryology*, *Carnegie Publications*, 16:163, 1925.
29. Sabin, F. R.: Bone Marrow, *Phys. Rev.*, 8: 191, 1928.
30. Sabin, F. R., Cunningham, R. S., Doan, C. A., Kindwall, J. A.: The Normal Rhythm of the White Blood Cells, *Bull. Johns Hopkins Hosp.*, 37:14, 1925.

31. Shew, A. F. B.: The Diurnal Tides of Leukocytes of Man, Jour. Path. and Bact., 30: 1, 1927.
32. Warner, D.: Diurnal Leukocyte Tide of the Rat, Proc. Soc. Exper. Biol. and Med., 33: 230, 1935.
33. Ames, D., and Huntley, A. A., The Nature of Leukocytosis Produced by Nucleinic Acid, J. A. M. A., 29: 472, 1897.
34. Vaughan, V. C., Novy, F. G., and McClintock, C. T., Med. News, 62: 536, 1893.
35. Wells: Leukocytosis and Immunity with a Critical Analysis of Nuclein Therapy, Med. News., 65: Oct. 17, 1896.
36. Tomlinson, O. R.: Nuclein Solution, J. A. M. A. 29: 478, 1897.
37. Doan, C. A.: The Neutropenic State, J. A. M. A. 99: 194, 1932.
38. Metchnikoff: Ann. de l' Institut Pasteur, 6, 1892. (Quoted by Ames and Huntley, Ref. 33.).
39. Buchner; Arch. f. Hugiene, B. 10 (Quoted by Ames and Huntley, Ref. 33.).
40. Weiskotten, H. G.: The Normal Life Span of the Leukocyte, Amer. Jour. Path., 6: 183, 1930.
41. Hueper, W. C.: Agranulocytosis (Schultz) and the Agranulocytic Symptom Complex, Arch. Int. Med., 42: 893, 1928.
42. Hueper, W. C., and Garrison, L. E.: Agranulocytosis, Surg. Clin. North Amer., 10: 407, 1930.
43. Weiss: Wien. Arch. f. inn. Med., 14:303, 1927.(Quoted by Hueper and Garrison, Ref. 42).
44. Zadek; Med. Klin., 21:688, 1925. (Quoted by Hueper and Garrison, Ref. 42).
45. Pelnar: Malignant Leukopenia, Cas. Lek. Cesk., 63: 1653, 1924, (Abstr. J. A. M. A., 84: 74, 1925).
46. Zikowsky: Wien, Klin, Wchnschr., 77:587,1927. (Quoted by Hueper and Garrison, Ref. 42.).
47. Fried, B. M. and Dameshek, W.: Exerpimental Agranulocytosis, Arch. Int. Med., 49:94, 1932.
48. Piersol, G. N., and Steinfield, E.: Granulopenia (Granulocytopenia), Arch. Int. Med., 49: 578, 1932.

49. Skiles, J. H.: Agranulocytosis, M. A. M. A., 84:364, 1925.
50. Pietti, E. C.: Histopathology of Agranulocytic Angina, J. A. M. A., 84:1415, 1925.
51. Bantz, R.: Leukemic Conditions with Extreme Leukopenia, Med. Klin. 19: 1667, 1923. (Quoted by Kastlin, Ref. 62.).
52. Moore, J. A., and Wieder, H. S.: Agranulocytic Angina, J. A. M. A., 85:512, 1925.
53. Cannon, A. B.: Unusual Dermatoses, South, M. J., 20:141, 1927.
54. Elkeles, A.: Agranulocytic Angina, Med. Klin., 20: 1614, 1924. (Quoted by Kastlin, Ref. 62.).
55. Schultz, W., and Jacobwitz, L.: Agranulocytosis, Med. Klin., 21: 1642, 1925. (Quoted by Kastlin, Ref. 62.).
56. Ehrmann, R., and Preuss, J.: Leukopenia in Sepsis, Klin. Wchnschr., 4:267, 1925. (Quoted by Kastlin, Ref. 62.).
57. Hunter, R. J.: Agranulocytic Angina--Case with fracture of the Tibia, Laryngoscope, 36:348, 1926.
58. Roberts, S. R., and Kracke, R. R.: Agranulocytosis, J. A. M. A., 95: 780, 1930.
59. Doan, C. A.: The Neutropenic State, J. A. M. A. 99: 194, 1932.
60. Jackson, J. Jr., Agranulocytic Angina and Allied Conditions. International Clinics, 3:69, 1934.
61. Pepper, O. H. P.: Leukopenia--A review; with special reference the agranulocytic angina, Calif. and West. Med., 35:173, 1931.
62. Kastlin, G. J.: Agranulocytic Angina, Amer. Jour. Med. Sci., 173: 799, 1927.
63. Stocke, A.: Folia Haemat., 40: 40, 1930. (From Beck, Ref. 2)
64. Dameshek, W. and Ingall, M.: Agranulocytosis (Malignant Neutropenia). Amer. Jour. Med. Sci., 181: 502, 1931.
65. Rosenthal, N.: Hematologic Aspects of Agranulocytosis and Other Diseases Accompanied by Extreme Leukopenia, Amer. Jour. Clin. Path., 1: 7, 1931.
66. Dennis, E. W.: Experimental Granulopenia Due to Bacterial Toxins Elaborated in Vivo, Jour. Exper. Med., 57:993, 1933.

67. Kracke, R. R.: A Review of Granulocytopenia, Jour. Lab. and Clin. Med., 17: 993, 1932.
68. Schilling, V.: The Blood Picture and Its Clinical Significance, Mosby, St. Louis, 1929.
69. Bromberg, L., and Murphy, P.: Agranulocytic Angina Following Prophylactic Typhoid Vaccination, J. A. M. A., 92: 1266, 1929.
70. Kracke, R. R.: Recurrent Agranulocytosis, Amer. Jour. Clin. Path., 1: 385, 1931.
71. Kopelowitz, J. C.: Agranulocytic Angina. Missouri State Med. Assoc. Jour., 26: 320, 1929.
72. Miller, D. K. and Rhoads, C. P.: Experimental Production in Dogs of Acute Stomatitis, Associated with Leukopenia and a Maturation Defect of the Myeloid Elements of the Bone Marrow, J. Exper. Med., 61: 173, 1935.
73. Day, P. L., Langston, W. C., and Shukers, C. F.: Leukopenia and Anemia in the Monkey Resulting from Vitamin Deficiency, J. Nutrition, 9: 637, 1935.
74. Moore, C. V., and Bierbaum, O. S.: Chronic Neutropenia Treated by Splenectomy, Internat. Clin., 3: 86, 1939.
75. Wiseman, D. K., and Doan, C. A.: A Newly Recognized Granulopenic Syndrome Caused by Excessive Splenic Leukolysis and Successfully Treated by Splenectomy, Proc. Amer. Soc. Clin. Invest. 1939, 18: 473,
76. Reisman, K.: Med. Welt., 12: 16, 1938. (From Moore and Bierbaum, Ref. 74.)
77. Thompson, W. P.: Observations on the Possible Relations Between Agranulocytosis and Menstruation, with Further Studies on a case of Cyclic Neutropenia, New Eng. Jour. Med. 210: 176, 1934.
78. Jackson, H. Jr., and Merrill, D.: Agranulocytic Angina Associated with the Menstrual Cycle, New Eng. J. Med. 210:175, 1934.
79. Corey, E. L. and Britton, S. W.: Blood Cellular Changes in Adrenal Insufficiency, and the Effects of Cortico-adrenal Extract, Amer. Jour. Phys., 102: 699, 1932.
80. Hubble, D.: The Influence of the Endocrine System in Blood Disorders, Lancet, 2: 113, 1933.
81. Kunde, M. M., Gree, F. M., and Burns, G.: Blood Changes in Experimental Hypo and Hyperthyroidism, Amer. J. Physiol., 99: 469, 1932.

82. Reznikoff, P.: Acute Neutropenia, *Internat. Clin.*, 3: 106, 1939.
83. Kracke, R. R. and Garver, H. E.: *Diseases of the Blood and Atlas of Hematology*, Phila., J. D. Lippincott, 1937-p. 138.
84. Martland, H. S.: The Occurrence of Malignancy in Radio-Active Persons, *Amer. J. Cancer*, 15: 2435, 1931.
85. Hoff, F.: Blut and Vegetative Regulation, *Erg. d. inn. Med. u. Kinderhik.*, 33: 195, 1928. (Quoted by Rosenthal, Ref. 65).
86. Muller, E. F.: Leukocytenstuz infolge Imspezifischer Intrakutanimpfung, *Munchen Med. Wehnschr.*, 69: 1506, 1922. (Quoted by Rosenthal, Ref. 65).
87. Muller, E. F., and Holscher, R.: *Ztschr. f. d. ges. Exper. Med.*, 38: 478, 1924. (Quoted by Rosenthal, Ref. 65).
88. Plum, P.: *Clinical and Experimental Investigations in Agranulocytosis with Special Reference to the Etiology*, Translated by H. Anderson, Lewis, London, 1937. (Quoted by Kracke, Ref. 105).
89. Kracke, R. R., and Parker, F. Jr., The Etiology of Granulopenia (Agranulocytosis) with Particular Reference to drugs containing the Benzene Ring, *Am. J. Clin. Path.* 4:453, 1934.
90. Jackson, H. Jr., The Differential Diagnosis of Agranulocytic Angina from Acute Leukemia, *Am. J. M. Sci.* 88: 604, 1934.
91. Rosenthal, N. *Agranulocytosis and Granulocytopenia*, Downey's Handbook of Hematology, New York. Paul B. Hoeber, Inc. 4: 2820, 1938.
92. Selling, L: A Preliminary Report of Some Cases of Purpura Hemorrhagica due to Benzol Poisoning, *Bull. Johns Hopkins Hosp.* 21: 33, 1910.
93. Selling, L.: Benzol as a Leukotoxin: Studies of the Degeneration and the Regeneration of the Blood and Hematopoetic Organs, *Bull. Johns Hopkins Hosp.*, 27: 83, 1916.
- 93A. Kracke, R. R. Experimental Production of Agranulocytosis, *Amer. Jour. Clin. Path.*, 2: 11, 1932.
94. Climenko, F. R., Inhibition of Leukogenic Activity in the Rabbit by certain Cyclic Compounds. *Proc. Soc. Exper. Biol & Med.*, 32: 823, 1935.

95. Madison, F. W., and Squier, T. L.: The Etiology of Primary Granulocytopenia, *J. A. M. A.*, 102: 755, 1934.
96. Benjamin, J. E., and Biederman, J. B.: Agranulocytic Leukopenia (Novaldin) induced by a drug Related to Aminopyrine. *J. A. M. A.* 107: 493, 1936.
97. Kracke, R. R., and Parker, F. P. Jr., Etiology of Granulopenia with particular reference of drugs Containing the Benzene Ring. *Jour. of Lab. and Clin. Med.* 19:799-815. May, 1934.
98. Sturgis, C. C., and Isaacs, R., Observations Concerning the Etiology of Agranulocytosis, *Tr. Assoc. Am. Phys.* 49: 328, 1934.
99. Dameshek, W., and Colmes, A. Effect of Drugs in Producing Agranulocytosis, with particular reference to Amidopyrine.Sensitivity. *J. Clin. Invest.* 15: 85, 1936.
100. Herz, L. F.: The Role of Amidopyrine in the Etiology of Granulocytopenia, with special reference to its Chemical Structure, *Jour. Lab. and Clin. Med.*, 20:33, 1934.
101. Davis, J. S., and Frissell, L. F.: Amidopyrine Hypersensitivity, *J. Lab. & Clin. Med.* 23: 107, 1937.
102. Rawls, W. B.; Effect of Amidopyrine upon the Red, White, and Polymorphonuclear Blood Cells, *Am. J. Med. Sci.* 192: 175, 1936.
103. Hoff, A.: Agranulocytosis following the Prolonged use of Allonal, *Minn. Med.* 20: 355, 1937.
104. Council on Pharmacy and Chemistry: The relation of the Amidopyrine and the Barbituric Acid Derivatives to Granulocytopenia, *Jour. A. M. A.*, 102: 2183, 1934.
105. Kracke, R. R.: Relation of Drug Therapy to Neutropenic States, *J. A. M. A.* 111: 1255, 1938.
106. Davidson, E. N., and Shapiro, M.: Neutropenia Following Dinitrophenol with Improvement after Pentonucleotide and Leukocytic Cream. *J. A. M. A.* 103: 480, 1934.
107. Bohn, S. S.: Agranulocytic Angina Following Ingestion of Dinitrophenol. *Jour. Am. Med. Assoc.* 103: 249, 1934.
108. Dameshek, W., and Gargill, S. L.,: Studies in Agranulocytosis-Report of 2 Cases Following use of Dinitrophenol. *New. Eng. J. Med.* 211: 440, 1934.



109. Silver, S.; Dinitrophenol Poisoning. J. A. M. A., Oct. 6, 1934, p. 1058.
110. Imerma, S. W., and Imerman, C. P.: Dinitrophenol Poisoning, J. A. M. A. 106: 1085, 1936.
111. Goldman, A. & Haber, M.: Acute Complete Granulopenia with death due to Dinitrophenol Poisoning. J. A. M. A. 107: 2115, 1936.
112. Farley, D. I.: Depressed Bone Marrow Function from the Arsphenamines, Amer. Jour. Med. Sci., 179: 214, 1930.
113. Blew, C. L.: A Report of three Cases of Agranulocytosis. U. S. Nav. M. Bull. 35: 484, 1937.
114. Groen, J., and Gelderman, C. J.: Agranulocytose (Maligne Neutropenia) door Due Geneesmiddelen, Nederl. tijdschr. v. geneesk., 78: 3444, 1934. (Quoted by Kracke, Ref. 105.)
115. Watkins, C. H.: The possible Role of Barbiturates and Amidopyrine in the Causation of the Leukopenic State. Proc. Staff. Meet. Mayo Clin. 8: 713, 1933.
116. Hudnutt, O. D.: Case of Agranulocytosis with Recovery Following the Administration of Concentrated Liver Extract. J. Mich. M. Soc. 34: 440, 1935.
117. Kracke, R. R., & Parker, F. Jr.: The Relationship of Drug Therapy to Agranulocytosis. J. A. M. A. 105: 960, 1935.
118. Costen, J. B.: Agranulocytic Appearance of Early Pharyngeal Lesions. Ann. O. R. L. 42: 372-384. June, 1933.
119. Shapiro, S., and Lehman, L.: A Case of Agranulocytosis Following Ingestion of Cinchophen. Am. J. M. Sc. 192: 705, 1936.
120. Zia, L. S., and Forkner, C. E.: The Syndrome of Acute Agranulocytosis and its Occurrence as a Complication of Kala-Azar. Am. J. M. Sci. 188: 624, 1934.
121. Taylor, A. B.: Agranulocytic Angina Purpura and Tuberculous Laryngitis. Lancet. 2: 73, 1937.
122. Watkins, C. H.: Neutropenia following Administration of Anidopyrine. Trans. Amer. Acad. Opth. 39: 292.
123. Fitz-Hugh, T.: Etiology and Pathology of Agranulocytic Angina. Am. J. Clin. Path. 7: 524, 1937.

124. Young, C. J. : Agranulocytosis and Sulfanilamide. Frit. M. J. 2: 105, 1937.
125. Ives, R. F.: Hemolytic Streptococcic Angina with Agranulocytosis treated with Prontosil and Sulfanilamide. Ann. Int. Med. 12: 882, 1938.
126. Jonston, F. D.: Granulocytopenia following Sulfanilamide Compounds. Lancet. 2: 1044, 1938.
127. Osgood, E. A.: Mode of Action of Sulfanilamide. J. A. M. A. 110: 349, 1938.
128. Lloyd, V. E., and Erskine, D.: Chemotherapy of Gonorrhoea with M & B 693. Lancet 2: 1160, 1938.
129. Johnston, F. D.: Agranulocytosis Following Treatment with M & B 693. Lancet 2: 1200, 1938.
130. Coxon, R. V., and Forbes, J. R.: Agranulocytic Angina Following Administration of M & B 693. Lancet 2: 1412, 1938.
131. Dolgopol, V. B., and Hobart, H. M.: Granulocytopenia in Sulfipyridine Therapy. J. A. M. A. 113: 1012, 1939.
132. Rosenthal, N., and Vogel, P.: Agranulocytosis caused by Sulfapyridine in Children, J. A. M. A. 113: 584, 1939.
133. Sheckel, H. A., and Price, A. E.: Fatal Granulocytopenia Following Sulfanilamide. J. A. M. A. 112: 823, 1939.
134. Shullenberger, W. A.: Agranulocytic Angina Following Treatment of Bacterial infections with Sulfapyridine. J. Ind. M. A. 32: 415, August, 1939.
135. Barnett, H. L.: The Treatment of Pneumococcic Infections in Infants and Children with Sulfapyridine. J. A. M. A. 112: 518, 1939.
136. Jaffe, R. H.: The Bone Marrow in Agranulocytosis, Arch. Path., 16: 611, 1933.
137. Isaacs, R: The Bone Marrow in Agranulocytosis, Arch. Path. 12: 142, 1931.
138. Jackson, H. Jr., and Parker, J. Jr.: Agranulocytosis: Its Etiology, and Treatment. New Eng. J. Med. 212: 137, 1935.
139. Custer, R. P.: Bone Marrow in Agranulocytosis. Am. J. Med. Sci. 189: 507, 1935.

140. Schwartz, W. F.; Garvin, C. F., Koletsky, S.: Fatal Granulocytopenia from Sulfanilamide. J. A. M. A. 110: 369, 1938.
141. Darling, R. C., Parker, F., Jackson, H.: The Pathological Changes in the Bone Marrow in Agranulocytosis. Am. J. Path. 12: 1, 1936.
142. Heck, F. J.: Recurrent Granulocytopenia. Proc. Staff Meet. Mayo Clinic. 9: 200, 1934.
143. Embleton, D.: Rhythmical Agranulocytosis. Brit. M. J. 2: 1253, 1936.
144. Gordon, M. B., and Litvak, A. M.: Differential Diagnosis Between Diphtheria and Oral Lesions of Blood Dyscrasias, Med. Jour. and Rec., 131: 35 and 74, 1930.
145. Griffith, G. C.: A Pronounced Case of Agranulocytosis with Recovery, Med. Clin. North Amer., 16: 209, 1932.
146. Friedemann, U.: Deutsche Med. Wchnschr. 53: 2193, 1927. (Quoted by Hamburger.).
147. Friedemann, U., and Elkeles, A.: Deutsche Med. Wchnschr. 56: 947, 1930.
148. Call, M., Gray, B. H., and Hodges, F. M. : Agranulocytic Angina-Report of a Case with Recovery. Amer. Jour. Roentgenology and Radium Therapy. Vol. XX, NO. 6, Dec., 1928.
149. Taussig, A. E., and Schnoebelin, P. C.: Roentgen Therapy in Agranulocytic Angina, Jour. A. M. A., 97: 1757, 1931.
150. Gager, L. T., and Speer, L. J: Roentgen Treatment of Agranulocytosis, Amer. Jour. Roent., 27: 40, 1932.
151. Harkins, H. M.: Granulocytopenia and Agranulocytic Angina with Recovery. Arch. Int. Med. 47: 408, 1931.
152. Marberg, C. M., and Wiles, H. O.: Yellow Bone Marrow Extracts in Granulocytopenia. J. A. M. A. 109: 1965, 1937.
153. Foran, F. L.: Agranulocytic Angina; a Case Report of Recovery Following Treatment by Intravenous Injection of Liver Extract. Ill. Med. J. 61: 521, 1932.
154. Foran, F. L., Sheaff, A. M., and Trimmer, R. W.: Agranulocytic Angina: Treatment by the use of Parenteral and Oral Liver Extract. J. A. M. A. 100: 1917, 1933.

155. Reich, C., and Reich, E., Further Studies in the Treatment of Agranulocytosis. J. Lab. & Clin. Med. 22: 503, 1937.
156. Strumia, M. M.: The Effect of Leukocytic Cream Injections in the Treatment of the Neutropenias. Am. J. M. Sc., 187, 527, 1934.
157. Reznikoff, P: Treatment of Agranulocytosis, Jour. Clin. Invest., 12: 45, 1933.
158. McLester, J. S., and Parsons, W. H.: Treatment of Granulopenia with Nucleotide Derivatives. Tr. Assoc. Am. Phy. 47: 204, 1932.
159. Doan, C. A.; Zerfas, L. G., Warren, S., and Ames, O.: Leukopenic and Leukocytic States, Jour. Exper. Med. 47: 403, 1928.
160. Jackson, H. Jr., Parker, F. Jr., Rinehart, J. F., Taylor, F. H. L: Studies of Diseases of the Lymphoid and the Myeloid Tissues, J. A. M. A. 97: 1436, 1931.
161. Jackson, H. Jr., Parker, F. Jr., and Rinehart, J. F.: The Treatment of Malignant Neutropenia with Pentose Nucleotides, Forty-seventh meeting of the Assoc. of Amer. Phys., Atlantic City, May 4, 1932.
162. Hamburger, L. P.: Angina Agranulocytica and Its Treatment, Bull. Johns Hopkins Hosp., 48: 333, 1931.