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Review of the Pelger-Huet anomaly and a study of the response of the Pelger cell to inflammation

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A REVIEW OF THE PELGER HUET ANOMALY AND A STUDY OF THE RESPONSE OF THE PELGER CELL TO INFLAMMATION

BY

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Introduction

The Pelger-Huet anomaly is known by a variety of other terms (10): "Pelger's Syndrome," "Pelger's anomaly," "False shift to the left," or "Familial false shift to the left of the leukocytes." (22)

It is an anomaly "characterized by a partial or complete failure of segmentation of the nucleus of the mature polymorphonuclear cells, with an increased clumping and condensation of the chromatin." (6) The peripheral smear of a Pelger-Huet individual can be misconstrued to represent a shift to the left if one doing the differential is not aware of the anomaly. The hyposegmented, mature granulocytes are recorded frequently as band cells, metomyelocytes, and even myelocytes. If one is aware the anomaly exists, it is hard to make this error. Undritz terms the blood pattern "pseudo regenerative."

Discovery

In 1928, Doctor Pelger (32), a Dutch physician and specialist in tuberculosis, described an anomaly of "leukocyte maturation" in two patients suffering from tuberculosis. He described the cell as characterized by the presence of an oval, indented, or bilateral nucleus in the neutrophil polymorphonuclear leukocytes which replaces the normal nuclear segmentation of these cells (2). A large percentage of these cells were stab forms. The rest were two segmented forms, while a few showed three segments but no more than three. The nuclear chromatin was coarse, grouped into irregular clumps, and obviously quite mature (22).

Doctor Pelger believed the leukocytes indicated a poor prognosis in this particular disease, and he did not think it to be an independent

anomaly. In 1931, he saw another patient with tuberculosis and "Pelger" cells. Thus the condition first became associated with this disease (22).

In 1931, G. J. Huet (18), a Dutch pediatrician, described the hereditary nature of the anomaly. He wrote of a ten-year-old female patient suspected of having tuberculosis. However, she did not have the disease. She did have the Pelger anomaly, and was a niece of one of Doctor Pelger's patients. He traced the family history back three generations and found elders possessing the same blood picture who were in good health (22). He concluded that no pathologic conditions could be associated with the blood picture. He then suggested it was an autosomal, mendelian dominant characteristic, non sex-linked.

Schilling subsequently proposed that the condition be called the Pelger-Huet anomaly (22).

Characterization of the Cell in the Peripheral Blood and in the Bone Marrow

The cells are characterized by decreased segmentation of the nucleus of the granulocytes (27). The unsegmented leukocytes are smaller than normal and appear in odd shapes...a dumbell, peanut, or rod (10).

The Pelger nucleus is generally smaller than a normal nucleus in relation to the cell (22). It is two-thirds the size of a normal nucleus (33).

The most common appearance of the nucleus in the Pelger-Huet anomaly is the two-lobed variety. No lobes in a relatively round nucleus is

the next most common type. Rarely, three lobes are encountered. The non-lobed nucleus may be round, oval or resemble the stab form (27).

A common variant of the two-lobed variety is the "pince-nez" form. It consists of two, rounded, symmetrical lobes connected by a thin filament. Occasionally the two lobes will be asymmetrical, or oval, or one round and one oval. These are not, strictly speaking, pince-nez nuclei.

Some of the cells with round nuclei (4) show chromatin threads radiating out from the nuclei into the cytoplasm. These are called "Stodtmeister cells." (51).

The connection between lobes can thicken to the point of presenting a picture of a dumbell. Similar cells have been termed "pseudomyelocytes." (49) The bone marrow has been termed "pseudomyelocytic." (35)

In the normal blood smear one sees nonsegmented nuclei in 3 to 5 percent of the neutrophils, two segments in 15 to 20 percent, three to four segments in 70 to 75 percent, and more than four segment in 5 percent. In Pelger-Huet there is an arrest of segmentation at the two lobe level (22).

Eosinophils are altered in the same fashion as the neutrophils, although this is not as conspicuous because normally 75 percent of the eosinophils have two lobes, and about 25 percent have more than two.

The basophils are similarly involved. Because of their low count, and usual low segment count, the anomaly is not generally recognized in basophils (22).

Skendzel (44) feels there is no abnormality in the lymphocytic or monocytic series. Others feel these series are affected (27).

Erythroblasts and megakaryocytes are also affected (33).

The chromatin is coarsely clumped. It is condensed markedly in the granulocytes, lymphocytes, monocytes, megakaryocytes, and normoblasts (27). It stains deeply with the basic dyes. The eosinophils, monocytes, and the lymphocytes are markedly affected in the homozygous state, but in the heterozygous state the nuclear abnormality is variable and sometimes slight (27). Post nuclear chromatin masses are visible frequently in the neutrophils (35). The chromatin appears very coherent. Molded clumps and chromatin dots are often in the blunt ends of the nuclei. Pyknosis of the chromatin may be present (22).

Maturation of the cell proceeds without the normal segmentation of the nucleus (22). Cytoplasmic granulation remains normal even when the abnormality is most marked (27). The cytoplasm/nucleus ratio has shifted in favor of the cytoplasm (35). Vacuoles are sometimes seen (22).

The cells in the marrow reveal the anomaly also. However, the anomaly does not extend to myeloblasts and promyelocytes (35).

Bone marrow examination (22) of the heterozygote shows increased numbers of myelocytes, metamyelocytes, and band forms and a lack of highly segmented granulocytes which usually account for a third of the granulocyte population. Instead of the typical lighter color of younger cells, the nuclei of the myelocytes are dark and coarsely

pitted. The chromatin tends to clump in the center of the nucleus. The nucleus is frequently at the periphery of the cell and is usually small in comparison to the cytoplasm.

Bone marrow examination from a patient, homozygous for the Pelger-Huet anomaly, shows a marrow rich in cells with a normal red/white cell ratio. The myeloid series is normal until the development of the younger myelocytes. The "chromatin begins to clump into dark, coarse corpuscles and stab formation was almost absent." (4) Some elongated nuclei were found. The cytoplasm matured normally. The lymphocytes and monocytes had the abnormal nuclear structure seen in the peripheral blood. In the red cell series the younger cells were normal, but in the basophil normoblasts, abnormal coarse chromatin clumps were observed. In the orthochromatic normoblasts the nuclei were pyknotic. Many of the megakaryocytes also had round nuclei.

The total peripheral white blood count is normal, while the differential count shows a significant shift to the left (22).

The anomaly is a non-reversible phenomenon (49). However, Undritz feels there are three situations in which the Pelger-Huet phenomenon may appear to reverse, or disappear. In these situations one could examine the blood smear and not recognize the Pelger-Huet anomaly. The situations which can "reverse" the blood picture are infections, intoxications, and allergy (49). During these states the blood would appear normal. After the removal of the cause of the reversal, the Pelger-Huet picture will again dominate.

Harm's Classification

Several systems have been developed to more quantitatively describe the anomaly. Harm's system (39,42) appears to be the most commonly used and referred to in the literature.

The neutrophils are the basis of the classification scheme. They may be divided into three types:

Type A	Normal
Type B	Intermediate
Type C	Typical Pelger-Huet

Type C cells have fewer nuclear lobes. Also there is a predominance of unsegmented nuclei and those with more than two nuclear lobes are rare. Nuclear chromatin of these cells is very basophilic, closely clumped, and gives a pitted appearance. The nuclear outline is smooth. The nuclei of the unsegmented cells are round, ovoid, or short, thick, plump rods. Bilobed cells are either symmetrical with lobes of equal areas or asymmetrical with one round or one ovoid lobe. The most characteristic and diagnostic cell is the "pince-nez" form.

Type B cells exhibit the above characteristics to a lesser extent. They tend to appear as normal cells. The chromatin is less densely clumped and the nuclear forms are less characteristic and smooth in outline. These cells make up 0 to 15 percent of the neutrophils in normal smears. They make up 0 to 30 percent in persons with Pelger-Huet anomaly.

Heredity

Huet (1931-1932) first suggested the hereditary nature of the anomaly and its transmission as a dominant, non sex-linked characteristic (6).

However, before discussing the hereditary aspects of the anomaly, it should be pointed out that there also exists a non hereditary form of the anomaly, referred to as the pseudo-Pelger anomaly. It is found in certain disease states or intoxications, and will be discussed later.

It has been fairly well established that the "familial" anomaly is inherited as a non sex-linked, Mendelian dominant characteristic (29). It has been reported to have been inherited recessively in at least one article (1). Its penetrance is thought to be almost 100 percent (22).

The defect can arise spontaneously as a mutation (20). Patau and Nachtsheim have estimated the mutation rate to be 1:10,000 (22,30). Undritz feels spontaneous mutation is extremely unlikely (49).

In 1939, Undritz discovered a similar anomaly in rabbits. Experimental transmission of the anomaly was now made possible in the rabbit (6). Nachtsheim demonstrated the anomaly in the rabbit to behave as a simple Mendelian with complete penetrance, independent of sex (29). A homozygous condition was discovered in which the nuclei ~~of the condition was discovered in which the nuclei~~ of the neutrophils were round or oval, and very few were bilobed. This was obtained by the mating of heterozygotes.

In addition to the homozygous and heterozygous forms (both "volltrager"), a third type of blood picture was described by Undritz (48) in 1937. He called this the "Teiltrager" or "partial carrier."

He stated that in these individuals a relatively constant proportion of the leukocytes are typical "Pelger cells," while the remaining leukocytes are normal (6,51). He reviewed this individual many years later (33). The patient had 80 percent normal and 20 percent Pelger cells for ninteen years. No relatives exhibited the anomaly. The question of illegitimacy is considered. However, this person could represent a new type of blood picture which is hereditary, and he could have mutated spontaneously. More likely he represents a mosaic. In 1937, Undritz thought perhaps children of these carriers might carry the full syndrome. He also thought perhaps this was an argument for incomplete penetrance.

In 1954 a second partial carrier was described by Stodtmeister and Undritz (49). In this case 80 percent are Pelger cells while 20 percent are normal. In this case, the grandfather, father, and son had the anomaly. It appeared to be a truly heritable condition. This is evidence that it might represent a separate entity, and not a chance mutation resulting in a mosaic. Harm's cell types A, B and C are all present simultaneously. Three percent of the nuclei were true Stodtmeister cells.

A third "Teiltrager" was described in 1957 by Petzel (33). Nearly all the Pelger-Huet cells of this individual were homozygous. The Pelger-Huet cells comprised 20 percent of his total neutrophils. Very few were indented or bisegmented. Many were Stodtmeister cells. The hereditary nature of this case has not been established.

Homozygotes

In the homozygous condition there is an extremely strong "suppression" of indentation. The basophilic chromatin is more coarse (33).

Nachtsheim (29) obtained the homozygous rabbit by mating of two heterozygotes. He did not succeed for a time. The 25 percent expected homozygous rabbits were born dead or would die shortly after birth. After breeding several litters, he obtained homozygotes which lived for several months. These animals were severely malformed. The long bones and the ribs were affected. He described it as a chondrodystrophy. One animal had micropthalmia. Others showed scab formation around the nose, salivation, and extreme emaciation.

The blood picture of the homozygous rabbits was deficient from the heterozygote rabbits. This fact shows the dominance is not complete. The heterozygotes present an intermediate form between the homozygotes and normal individuals. The heterozygotes show rod and spectacle forms of nuclei. The homozygotes show round nuclei and a very coarse chromatin structure resembling those of certain primitive vertebrates such as alligators, turtles, and snails, and in the invertebrate animals. The coarse chromatin structure is also shown by the lymphocytes and monocytes, and the monocytes are less indented than normal (4).

Begemann and Campagne (4) described in 1957 a human homozygote. She was a two and a half year old girl, first seen for epilepsy. She was born to heterozygote parents. Ninety-five percent of her neutrophils

had round nuclei. The mother had three children die at an early age, and two abortions. The conclusion one would be tempted to make is that the homozygous state is lethal in most cases. However, the parents are first cousins, and other defects could have been the cause of the deaths and abortions. Several other relatives had epilepsy. X-rays of the skeleton showed no abnormalities. The investigators feel that this case demonstrated that the homozygous state is not necessarily lethal as it is in rabbits. The chance of a homozygote to appear in a population is 1:1,000,000 (33).

A second homozygous case was described in 1956 by Bernard et al (33). The patient was an Algerian soldier whose blood was first examined when he had a case of the flu. Blood and bone marrow studies were done. Unfortunately, the political situation was such that the parents could not be examined. Ninety-seven percent of the neutrophils had round nuclei. (Little detail is given in the article as to followup, but note that pseudo-Pelger has been reported in a person due to the flu) (1).

Incidence

Bilobed neutrophils indistinguishable from Pelger cells occur normally in the alligator, snake, turtle, fowl, anteater, sloth, and many other species (44,23).

In the United States, the incidence has been reported to be 1: 5,000 to 1:10,000 (27). Davidson (44) calculates the incidence to be 1:6,000. Shendzel (44) calculates the incidence to be 1:4785. He

points out the anomaly is five times as common as hereditary spherocytosis and is really not rare. It is probably frequently overlooked.

Ludden (25) reviewed blood smears from 43,000 people in an attempt to obtain a true incidence. Three of four individuals found were of German or Dutch extraction. They felt this was significant, especially in light of the high incidence reported by Nachtsheim.

Nachtsheim (29) studied routine blood smears. He concluded that taken at random, the incidence in human beings would be 1:1,000 equally distributed between male and female. This was based on studies of a population in Berlin of 20,000 people. Climate and racial background were of no significance. Undritz in 1943 thought incidence was 1:1,000 (33).

It has also been observed mainly in European countries. It has also been observed in inhabitants of the Orient (10). It has been reported in persons of Caucasoid, Oriental, and Negroid stock. The incidence is reported 1:6,000 in London (7). In Japan the incidence is reported 1:20,000 (49).

The greater incidence in the East European countries is probably related to the fact that since the anomaly was first reported there, and there is more interest in it there than here. Much literature on the subject is written in these countries.

The incidence in the United States is probably larger than reported or estimated at the present time, simply because the slight shift to the left can frequently be attributed to an infection. Differentials

are usually ordered when a person is ill, and a small shift to the left can be attributed to this. One must actually be aware of the anomaly and be looking for it, and screen a large number of "normal" subjects to get a true idea of the incidence.

In the subjects studied and reported in this paper, two with the anomaly were daughters of a physician, five were children of dentists, two were dentist's wives. These people were subjected to good medical attention and had individually many differentials for various reasons. Yet they were never diagnosed as Pelger-Huet until the study herein was undertaken. How many other people have this anomaly but are not yet diagnosed?

Pseudo-Pelger

In the literature there is some overlap as to what constitutes the pseudo-Pelger-Huet anomaly, and what constitutes true Pelger-Huet anomaly occurring by chance in an individual with another disease.

The anomaly has been reported in the literature in patients who simultaneously had elliptocytosis (39), megaloblastic anemia (2), myeloproliferative disorders (35), chronic myelocytic leukemia (1,17, 43,45), aleukemic myelosis (45), agranulocytosis (6,15), tuberculosis (18), mutliple cartilagenous exostosis (36), cystic fibrosis with hypogammaglobulinemia (34), diabetes mellitus and reticulum cell sarcoma (41). Actually, almost any entity could conceivably be reported in a person with Pelger-Huet anomaly.

Ardeman (2) describes an interesting case in which megaloblastic anemia occurred in a woman with Pelger-Huet anomaly. Before treatment with B_{12} the neutrophils showed three and four lobes. The sex chromatin body was present. After treatment began, the average lobe index dropped. In a month, the neutrophils looked Pelger. The sex chromatin bodies disappeared.

The true pseudo-Pelger anomaly is the "acquired" or "non-familial" (20) form of the anomaly. It is characterized by (27):

1. The absence of the anomaly in the parents or other members of the family.
2. The presence of disease states known to produce the anomaly.
3. "Pelgeroid cells" (3,6,14,35)

The anomaly is confined to the cell nucleus and manifests itself late in morphogenesis. It is thought to be due to an arrest or dysplasia of nuclear chromatin synthesis. The occurrence of pseudo-Pelger anomaly is indicative of a fundamental disturbance of chromatin synthesis. The exact mechanism is obscure. The disorder may be due to a number of intrinsic or extrinsic agents arresting or altering nuclear development. Whatever the mechanism, it is a relatively late event in nuclear foundation since the chromatin and cytoplasm of the Pelger cells have mature characteristics. "Dwarf lobes" are found in some of the neutrophils, suggesting congenital malformation (9).

In leukemia, as with other abnormal cells (i.e. micromyeloblasts, Reider cells, Turk cells, Ferrata cells) an asynchronous maturation

between cytoplasm and nucleus probably exists due to enzymatic derangement of nucleic acid metabolism.

In pseudo-Pelger states, it should be noted that there exists varying numbers of Pelger-Huet granulocytes in blood and marrow and these patients will have normal leukocytes in addition in varying numbers. As one develops the facility for recognizing Pelger-Huet cells, they will be noted more frequently in smears in which normal leukocytes predominate (42).

Pseudo-Pelger has been described in rats with chronic leukemia (16). Experimentally the pseudo-Pelger anomaly has been transmitted from one rat to another with the transfusion of myeloid chloroleukemia in these animals (16).

The following disorders have been known to cause the development of Pelgeroid cells in the blood of the patient:

1. Atypical leukemia (5)
2. Acute granulocytic leukemia (42)
3. Chronic granulocytic leukemia (most common) (6,27)
4. Myeloid metaplasia (9)
5. Myelotoxic metaplasia (9)
6. Agranulocytosis (6)
7. Multiple myeloma (42)
8. Myeloproliferative disorders (42)
9. Severe myxedema (43)
10. Francon's panmyelopathy (46)
11. Influenza (1)
12. Exanthem Subitum (8)
13. Malaria (27)
14. Lupus erythematosis (50)
15. Drugs (Gantrisin) (21)
16. Chemicals (benzene) (31)

The cells in the acquired anomaly and the familial form are identical morphologically. Some of the leukocytes in the acquired type

associated with myelocytic leukemia resemble the cells of the homozygous Pelger-Huet anomaly (27).

In most cases of pseudo-Pelger in chronic leukemia and myeloid metaplasia, the change occurred following prolonged exposure to myelotoxic therapeutic agents (9). In cases occurring in patients with acute leukemia, the pseudo-Pelger anomaly is usually noted before therapy is started.

Kaplan (21) reports on the pseudo-Pelger anomaly developing in a patient which Gantrisin was used to treat a pyelonephritis. After six days of treatment the patient developed a new fever, a maculopapular rash, and a peripheral smear showing the heterozygotic Pelger-Huet picture. A smear six weeks prior to treatment, and one six weeks after Gantrisin was stopped, showed normal neutrophils. They postulate that the alteration of nuclear development may be due to a hypersensitivity phenomenon rather than a direct toxic effect.

The case of chronic myeloid leukemia described by Darte (6) et al represents the typical picture of the pseudo-Pelger anomaly. The patient presented with leukocytes which had many of the characteristics of the Pelger leukocyte anomaly. As the disease progressed, the adult neutrophils progressed to rounded nuclei, with coarsely clumped chromatin simulating the homozygous type of Pelger cell. Familial Pelger-Huet anomaly does not show this progression. Limited family studies were negative for the anomaly.

Pathologic Physiology

The Pelger-Huet anomaly is apparently a completely harmless condition. (49). It is a mere morphologic deviation, comparable to ovalocytosis (22). Persons with the anomaly have been found in good health at all ages and their longevity has not seemed to be influenced by the anomaly. Leitner and Gugelot (24) studied the phagocytic activity of the neutrophils in an investigation concerning the question whether a person with Pelger-Huet is more susceptible to infection. They concluded that mature Pelger cells have the same functional capacity as normal mature white cells have, but that the immature Pelger neutrophils have less phagocytic activity (22). They suggested that in severe infection, when the peripheral blood contains many immature Pelger cells, the patient's defense mechanism is less than a normal person's. However, in reviewing the cases with Pelger-Huet anomaly, no increased incidence of infections of other illnesses was noted. Undritz (33) also feels there is no increased incidence of infections.

It has been shown (44) that the phagocytic activity of the mature Pelger cell is within normal limits, using a suspension of Staphylococcus aureus.

Nachtsheim (29) suggests that perhaps severe infections may be more detrimental to Pelgers than non-Pelgers.

Rauchfuss (36) reported the first case of multiple cartilagenous exostoses associated with Pelger-Huet anomaly in 1959. It appears this was just chance that the two anomalies occurred together.

The leukocytes are reported to retain their typical features in inflammatory exudates (28). Supravital staining with Janus-green and neutral red showed cells displaying the normal movement.

Klein (22) reported the first autopsy on a Pelger. He found no positive findings attributed to the anomaly.

No correlation of the Pelger-Huet defect with blood type has been demonstrated (28).

Cortisone will cause an elevation in the total white count in a Pelger-Huet individual, but will not change the anomaly. It will cause a shift to the left, however, (22).

The alkaline phosphatase activity of the Pelger cells is reported to be normal, determined by the modified azo-dye technique (44). This would tend to rule out the concept that they are an immature cell.

The pseudo-Pelger cells, homozygous or heterozygous, in myeloid metaplasia and myelogenous leukemia, show a strong cytoplasmic alkaline phosphatase using the Gomori calcium cobalt method (9).

The Graham-Knoll peroxidase reaction was strongly positive in pseudo-Pelger cells in myeloid metaplasia and myelogenous leukemia (9).

Pseudo-Pelger cells show normal cytoplasmic esterase activity using Naphthol ASD chloroacetate as a substrate (9).

In rabbits with the heterozygous Pelger trait it has been demonstrated that intravenous injections of colchicine results in the formation of rounded nuclei similar to those seen in the homozygous form of the anomaly (6,12,29).

Colchicine is a drug known to affect mitosis. Harm (12) feels his observations may be interpreted as a transient partial phenocopy of the Pelger gene.

Some similar subtle alteration in the nucleic acid metabolism is thought to be responsible for the changes in Pelger-Huet nuclei and also in the pseudo-Pelger anomaly such as is seen with chronic myeloid leukemia (6). The actual cause of this derangement is not known.

Theories for the etiology of the anomaly include:

1. Failure of proper development of the bone marrow
2. An abnormality of chromatin dispersion that resists segmentation (10)
3. Enzymatic deficiency

Report of a Case:

A 27-year-old white medical student was discovered to have had the heterozygous form of Pelger-Huet anomaly five years ago when a screening differential was done on admission to the University of Nebraska Medical School. Up to that date the patient had no idea that he might have a blood anomaly. He had several differentials done during previous years for physical examinations, yet the anomaly never was noted.

The patient states that he has never had significant illness in his life. On two or three occasions, he required drainage and antibiotics for cellulitis before entering college. While an undergraduate, he developed a serious cellulitis in his left leg which required a week of soaks, outpatient care and antibiotics to cure. While in medical school, he had three incidents which developed into infections requiring

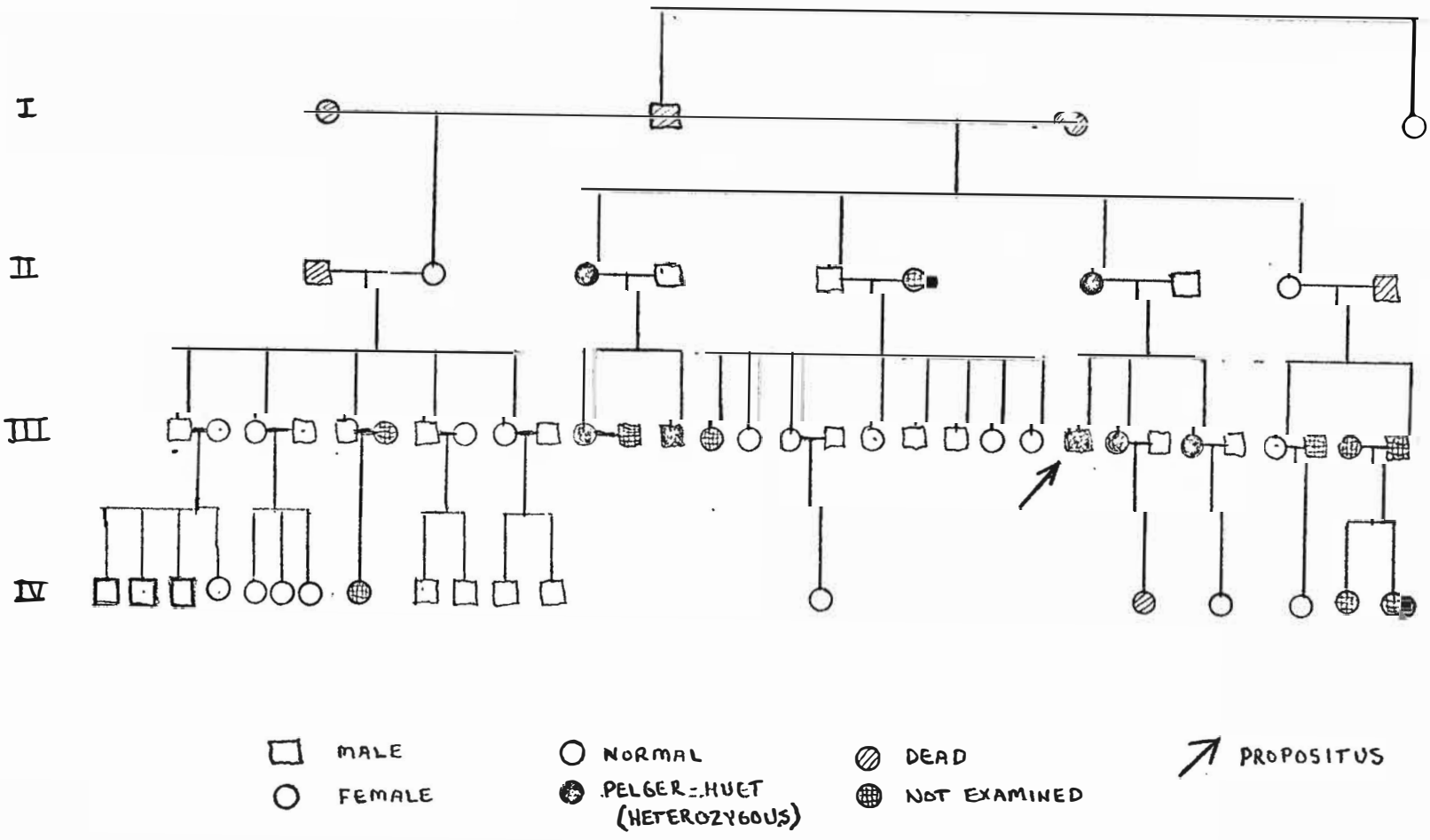
treatment at student health, antibiotics, and soaks. Two of these incidents caused the student to lose time in school. He states that he has never felt he becomes sick more often than other students, but he feels he has "colds" which last longer than they should. An average "cold" will last two to three weeks.

He also reports that he has broken several bones during his life. The nose has been fractured several times, a metacarpal, a phalanx, vertebral spinous process, and humerus. The humerus required eight months to unite. Calcium, phosphorus, acid and alkaline phosphatases were normal.

A pedigree was then done by this investigator on the patient's family. The results are shown on the chart on the succeeding page. The patient's mother and two sisters had the anomaly. A sister to the mother and her two children (male and female) also were found to have the anomaly.

Association with Factor XI Deficiency?

The health history for all the affected individuals was negative as far as could be ascertained, with two exceptions. The youngest sister of the propositus frequently had difficulty with menorrhagia. An extensive workup for clotting abnormalities revealed a probable Factor XI deficiency. This was based on a slightly increased partial thromboplastin time, and a partial correction of the patient's plasma by the addition of normal plasma and serum, but not saline.


I
II
III
IV
MALE
FEMALE
NORMAL
PELGER-HUET (HETEROZYGOUS)
DEAD
NOT EXAMINED
PROPOSITUS

Complete thromboplastin generation time was performed on the stored and frozen serum of the girl, but this was within normal limits. However, the patient was pregnant at this time, and this affects various coagulation factors. Also, Factor XI will increase upon storage. Therefore, these studies will be repeated.

A cousin to the propositus and his sister also reports menorrhagia. She has been seeking treatment for this condition for several years. Her complaints were very similar to the above mentioned girl. An investigation of her clotting factors is currently underway.

Passage of Pelger-Huet Cells from Blood Vessels into the Area of Inflammation

One of the questions which arises concerning Pelger leukocytes is whether they function normally. One of the functions of the neutrophils is to pass from blood vessels and migrate into an area of inflammation. They are one of the first leukocytes to reach the area of inflammation. There, they phagocytose bacteria and foreign substances. Eventually, they are replaced by other cells of the leukocytic series. A simple study would be to time the appearance and disappearance of the neutrophils in a Pelger-Huet individual and a normal person in an area of inflammation. These results could be compared with each other and be compared to known values obtained in checking large numbers of persons, measured the same way.

Materials and Methods: The "Rebuck Window" technique seemed to be appropriate for this study. Rebuck and Crowley (37) reported this as a technical procedure designed originally to evaluate the relative importance of the role played by lymphocytes and monocytes as macrophage producers in acute inflammation.

A modification of their technique was employed. The anterior surface of the thigh was chosen as the site. The skin was shaved and cleansed with alcohol. Then by means of an injector razor (cleansed with alcohol) the epithelium is scraped away from an area 3 or 4 mm. in diameter. When the papillary layer of the corium was reached, fine bleeding points were evident. The time of the removal of the epithelium constitutes the beginning of experimental inflammation. The trauma itself is an inflammatory stimulus.

The lesion is then immediately covered with a sterile cover slip. This is surmounted with a square of cardboard slightly larger than the cover slip. The cardboard is then taped securely to the leg. The center of the tape overlies the lesion. To obtain more tension, several gauze pads are placed over the center of the lesion, and these are taped securely. Standard sized cover slips were employed.

The cells of the inflammatory exudate migrate to the undersurface of the cover slips, and flatten themselves out as they do so. After a specified length of time, the cover slip is removed, and rapidly air dried. At the same time another cover slip is placed over the same lesion, and the process is repeated at timed intervals.

According to Rebuck this allows the obtaining of a series of fixed, permanent, preparations of *in vivo* samplings of the cellular exudates of man.

A single layer of exudative cells attach to the undersurface. Wright stain using standard technique is employed. The cover slips are stained first then mounted individually on slides using Permount.

One of the technical points which proved troublesome was the determination of the surface of the cover slip with the one layer of cells. It would be advisable to mark the top of the cover slip before removing it from the leg in any future series.

Another point to make the experiment easier would be to mount the slips first and stain later on slides, as one would stain blood slides.

The cover slips were applied to the subject mentioned previously with Pelger-Huet anomaly and a control using identical technique. The cover slips were removed after one-half hour, one hour, 2 hours, 6 hours, 14 hours, and 21 hours on each of the subjects.

The control subject was a white male of approximately the same age, height, and weight. Both subjects were in apparent good health.

The results of the two subjects were compared. Then both were compared to results obtained by Rebuck. The two significant parameters were (1) rate of appearance and disappearance of neutrophils, and (2) ratio of neutrophils to other cells.

Results: Comparisons between the control and the Pelger-Huet showed identical pictures. There did not seem to be any decreased time for migration of the Pelger neutrophils to the area of inflammation. Also, the results appeared to coincide with Rebuck's findings in the parameters measured above.

The neutrophils in the Pelger-Huet individual were the typical Pelger cells. A differential done on the neutrophils showed them to be in essentially the same ratio (bilobed, band, pince-nez, etc.) as in a peripheral smear.

The slips from the one-half, one and two hour specimens were essentially the same. No difference could be ascertained. The one-half hour specimen had increased numbers of red cells on it, in both the control and subject. There were approximately 80 percent granulocytes to 20 percent lymphocytes. The neutrophils predominated the slide. A few lymphocytes were present. A few tissue histiocytes were also present.

At 6 hours the neutrophils were still the most numerous of the cells. The ratio was about 60 percent neutrophils to 40 percent lymphocytes. The neutrophils were smaller than usual, and appeared to be degenerating. The cytoplasm was less granulated and scant. The nucleus was fragmented in most of the neutrophils. Occasional eosinophils were noted.

At 14 hours the neutrophils were in the minority. Many were degenerated. Rebuck states that about 25 percent of the cells present were degenerated neutrophilic remnants. This is in general agreement

with the results obtained in this experiment. Lymphocytes were much larger now. Cytoplasm was increased. Vacuoles were present in the cytoplasm. It appeared that the neutrophils were being ingested by the macrophages present.

At 21 hours Rebuck reports evidence of a second migration of neutrophils in moderated numbers. This was not observed in the present experiment. The predominate cell was the large lymphocyte and histiocyte.

Discussion: The parameters measured were actually vague. An attempt to quantitate the results by doing differential counts on the cell on the various cover slips was not very accurate. The reason is that the cells tend to clump in the periphery of the slips, in great quantities, and one can get different counts with each new high power field. Therefore, several differential counts were done and the results expressed in a crude average percent.

Also no conclusions can definitely be drawn on the result of one experiment on one Pelger-Huet individual. However, it is my impression the results were quite representative of any future experiments such as this.

A second invasion of neutrophils was noted by Rebuck at 21 hours. Perhaps this would have been noted in this experiment at a sooner or later time. Rebuck took slips at 20 intervals in 24 hours. He averaged his results for 250 patients for his descriptions at "21" hours (and

other times). A large number of repeated experiments such as he did might show this phenomenon in the Pelger-Huet individual and control used here.

Summary

1. Pelger-Huet neutrophils and eosinophils migrate to the scene of local inflammation, as do normal neutrophils and eosinophils.
2. Neutrophils of Pelger-Huet variety appear to migrate in approximately the same time from the onset of inflammation as do normal neutrophils.
3. They do not appear to degenerate in a more rapid period of time when once migrated to an area of inflammation.

In Conclusion:

"Superficially, the Pelger-Huet cells might be thought to be normal stab neutrophils or metamyelocytes. However, three things should alert the observer to the anomaly:

1. Coarseness of nuclear chromatin;
2. Pince-nez appearance;
3. Absence or scarcity of segmented forms." (27)

When there is doubt, the familial occurrence of the anomaly will confirm the diagnosis. However, failure to find the anomaly in other members of the family does not disprove the diagnosis. Genetic mutation could have occurred (22).

If a person thinks he has a slide from a Pelger-Huet, he should mentally rule out acute or chronic granulocytic leukemia. However,

these diseases produce a picture, with the Pelger cells constituting only a small number of the total neutrophils. There are usually always a certain significant percentage of 3 and 4 segmented neutrophils. Truly, immature granulocytes will also be seen (44).

A person examining a slide thought to be Pelger-Huet should also consider an acute infection. The above essential differences will occur in this situation also. The bilobed cells in an infection will also have finer nuclear chromatin, with elliptic and unequal lobes (44).

On the other hand, if a person is examining a slide with a suspected shift to the left, he should mentally rule out the Pelger-Huet anomaly. Patients could be treated unnecessarily for infections, exposed to unnecessary expenses for numerous diagnostic studies, or even subjected to unnecessary operations on the basis of their differential in certain situations. Unnecessary treatment could lead to iatrogenic disease. One member of a family described by Klein (22) had been under treatment for a "blood condition" for years.

Rosse and Gurney (39) discuss a patient who was thought to have appendicitis. The differential showed a characteristic shift to the left. However, the operation was avoided because the anomaly was recognized.

It is important then that a person with this anomaly be informed about its presence and its nature so he can inform his physician to its existence.

Klein, Hussar, and Bornstein (22) believe the evidence is such that two persons with the anomaly should not marry because of the probability of its being lethal in the homozygous state. No other investigators, to my knowledge, have ever gone so far as to make such an admonition.

The anomaly could conceivably be helpful in a case of disputed parentage since it is a dominant, non sex-linked characteristic (27,49).

The anomaly could be used in certain cases of legal identification (40).

The cells could be used as biologic tracers, and studies of leukocyte survival, life span, and intravascular residence time could be undertaken. Rosse and Gurney (39) used the anomaly as a tagging device to investigate the survival time of transfused neutrophils in peripheral blood. Most of the cells were absent from the peripheral blood in 6 to 8 hours. None were found after 49.5 hours. One of the problems encountered in determining the length of survival in peripheral blood of transfused leukocytes is tagging the exogenous cells so they may be distinguished from endogenous cells.

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