Holmes (A. M.)

Compliments of the Author

THE TECHNIQUE OF BLOOD-STUDY AND EXPERIMENTS IN THE PHYSIOLOGICAL CHEMISTRY OF LEUCOCYTES.

A STUDY IN CELL TISSUES AND THEIR SIGNIFICANCE IN TUBERCULOSIS.

BY

A. MANSFIELD HOLMES, A. M., M. D.

DENVER, COLORADO

LECTURER ON HÆMATOLOGY IN THE UNIVERSITY OF COLORADO; HÆMATOLOGIST TO ST. JOSEPH'S HOSPITAL; MEMBER OF DENVER AND ARAPAHOE AND COLORADO STATE MEDICAL SOCIETIES, ETC.

Read before the Second Pan-American Medical Congress, in the City of Mexico, November 17, 1896

REPRINT FROM THE MEDICAL RECORD, MARCH 13, 1897

DENVER, COLORADO
THE SMITH-BROOKS PRINTING COMPANY
1897

The Technique of Blood Study and Experiments in the Physiological Chemistry of Leucocytes—A Study in Cell Tissues and their Significance in Tuberculosis.[1]

By A. MANSFIELD HOLMES, A. M., M. D.,
Lecturer on Hæmatology in the University of Colorado; Hæmatologist to St. Joseph's Hospital; Member of Denver and Arapahoe, and Colorado State Medical Societies, etc.

Disease, like a living organism, develops from an invisible beginning. In other words, it develops from an invisible entity into a visible entity. It is generally believed that there is a latent stage in the development of all diseases. In many diseases this is known as the prodrome, which sooner or later develops into the active stage. Tuberculosis, in its development, is no exception to the general rule. In the so-called active stage of tuberculosis there are disintegration and wasting of tissues. Hence, it is customary to date the beginning of the active stage from the first apparent evidence of disintegration. If we trace tuberculosis backward from the stage of visible disintegration, we shall sooner or later reach a period in its development in which the evidence of disintegration disappears from the patient. We thus pass from the visible into the invisible realm. It is our purpose now to investigate this invisible realm. The microscope reveals the fact that the disease in this so-called latent stage is in progress, though not apparent to the senses. That which is understood as the predisposition is now seen in the blood elements as a condition. In the active stage of the disease we were studying the patient; now we are studying his leucocytes. And I find that the law that brings about disintegration in the larger organism has already brought about the same process in the leucocytes at an earlier date. Hence, I believe that I have shown that there is no latent stage of the disease. It is all active. Therefore I believe that which is termed the predisposition to disease is not latency. It is an invisible activity, which is either advancing unconsciously to the patient, toward visible activity, or convalescing toward the normal state.

[1]Read before the Second Pan-American Medical Congress, in the City of Mexico, November 17, 1896.

With this preliminary statement of the subject, I will proceed to give it more in detail. The material out of which I have formulated my deductions has been obtained from a study of more than one hundred cases. Hence, with nature's proof, I feel justified in claiming that in tuberculosis the condition of the individual can be ascertained from the appearance of the leucocytes of his blood.

The clinical study of disease in the larger animal organism has been reduced to a scientific technique. An important principle in the study of pathological conditions is based upon the fact that a change in the appearance of an organism is preceded by a change in its functions. Hence, to be able to distinguish disease from the appearance of tissues, it is necessary to become familiar with these tissues when their functions are normal. In other words, we must be familiar with normal histological conditions before we can distinguish pathological conditions. Therefore, the technique of the clinical study of disease in the larger animal organism or in its gross tissues is followed out according to uniform methods. The temperature, pulse, and respiration reveal the degree of functional activity. Auscultation, percussion and inspection reveal definite conditions of the organs. Chemistry determines the condition of the secretions and excretions. The family and personal histories help to reveal tendencies which may have been transmitted by the laws of heredity or acquired through the medium of uncongenial environment.

In a previous article,[1] I made the statement that the larger animal organism has a true prototype in the leucocytes of its own blood. Hence, since the condition of the larger organism can be ascertained through a careful and systematic technique, I therefore claim that the condition of the leucocytes can also be ascertained, if a proper technique is adopted.

A study of the blood requires an intimate acquaintance with cell tissues. And these are so delicate that the microscope and staining fluids must be brought into use before they can be distinguished. Hence, if disease can be recognized from the appearance of gross tissues, it should be equally true of cell tissues. Therefore, if an examination of the blood is made in any particular disease and repeated observations demonstrate that def-

[1] *Medical Record*, September 5, 1896.

inite blood conditions are uniformly present, the condition of the cell tissues should be accepted as evidence of diagnostic value, equal with the physical signs observed when studying the larger organism.

Furthermore, the tissues of the larger organism are constructed of and replenished by leucocytes that undergo differentiation. Hence, if it is possible to ascertain the condition of the leucocytes before they become differentiated into tissue cells, it will reveal the predisposition of the larger animal organism before the actual condition exists, or at least before it can be recognized by the natural eye. On the other hand, before we can scientifically utilize the appearance of leucocytes in the diagnosis of disease, it must be demonstrated that these conditions and appearances uniformly exist in the cells, and that they bear a definite relation to the larger organism. This, therefore, necessitates a study of cell tissues, and before studying them it will be necessary to review

THE TECHNIQUE.

In the ordinary study of blood an exact technique is unimportant. But, if it is our purpose to detect the physiological or pathological conditions of leucocytes from the delicate and peculiar reaction of their tissues to stains, it is of the greatest importance to adopt a technique that is accurate and uniform. This technique, then, should be adhered to in every detail, if we expect to secure results that will admit of comparison. The technique which I have adopted is divided into the following steps: (*A*) Preparing the blood films; (*B*) fixing; (*C*) staining; (*D*) mounting, and (*E*) studying the mounted specimen.

(*A*) Preparation of Films: This is by no means an unimportant factor. Unless it is properly performed, all subsequent work will be useless, and the specimen will lead to erroneous conclusions. In fact, a failure in any part of the work will render the specimen useless. The following points should be carefully observed: The cover glasses should be absolutely clean, dry and polished. They should be handled with forceps to prevent moisture from collecting on the surface, which prevents the blood from properly spreading. Fine lint fibres often act in the same manner. The finger of the patient is then cleaned antiseptically and pricked with a sterilized needle. For this purpose Emmet's cervical needle, with

triangular point, is quite satisfactory. The drop of blood should then be used immediately, and a fresh drop for each film. A cover glass held in forceps is touched to the apex of the drop of blood, the drop coming in contact with the center of the glass. This is immediately placed upon another cover glass. If the cover glasses are clean, dry and polished, the blood immediately begins to spread and continues uniformly in every direction. If the drop does not spread satisfactorily, it is evident that there were moisture, lint fibres, or foreign substance on one or both of the cover glasses. As soon as the spreading ceases, the cover glasses should be immediately separated by pulling them in parallel planes, in opposite directions. The blood spreads by capillary attraction, and in no case should pressure be used. Too much blood or not enough will give poor films. And when films are poorly prepared they should be destroyed immediately, and another trial should be made. An ideal film consists of a single layer of cells.

ARTIFICIAL INJURY TO CELLS.

Crushed corpuscles are often observed in the study of prepared specimens, and may result from two causes: (*a*) From a faulty technique; and (*b*) from a disintegration resulting from a feeble organization of the cell tissues. The first is the result of a post-mortem injury to the cell. The second is due to an ante-mortem disintegration, and always signifies a weak or diseased cell, endowed with feeble vitality. If the corpuscles are injured in the process of preparation, fragments and débris will always be seen uniformly extending from the injured cell, in the opposite direction from which the cover glass was separated from its companion. A practiced eye readily distinguishes these varieties, and unless they can be so distinguished, erroneous opinions may be formed.

(*B*) Fixing: As a fixing solution, I use the following: Absolute alcohol and Squibb's ether, equal parts, to which I add 5 per cent. of distilled water. The uniform time for fixation is two minutes. I have observed that a freshly prepared fixing solution never gives results equally as good as an older solution. Fresh solutions have a tendency to crenate red corpuscles and to produce vacuolation in the hæmoglobin. I have also observed that the longer the solution is used, the better

are the results. I can account for this improvement only on the ground that the solution is rendered less injurious to the red cells by evaporation, the ether evaporating more quickly than the alcohol or water. Hence, when a new fixing solution is prepared, I allow it to stand for a few hours exposed to the air before using it. It is also important to note that in films fixed immediately after being taken, the red cells are less liable to crenate and the structure of the cells is more perfectly preserved.

(*C*) Staining: Almost all animal tissues possess great affinity for stains. This is true of cell tissues as well as of the gross tissues of the animal organism.

CLASSIFICATION OF DYES.

There are two important classes into which dyes are divided: 1, according to their chemical reaction; 2, according to their behavior to tissues. The first is subdivided into (*a*) acid, (*b*) basic and (*c*) neutral dyes. The second is subdivided into (*a*) substantive and (*b*) adjective dyes. Of the first class, the majority belong to the acid and basic groups. Neutral dyes are rare. Substantive dyes are absorbed by the tissue directly from the staining solution. Adjective dyes require the intervention of a mordant, which aids in staining the tissues by forming insoluble compounds with the coloring matter, or by serving as a medium on which the staining solution acts.

CLASSIFICATION OF TISSUES.

All tissues, animal and vegetable, may be classified according to their staining properties under four heads: (*a*) Oxyphile tissues react to acid stains; (*b*) basophile tissues react to basic stains; (*c*) amphophile tissues react either to acid or to basic stains, or to both; (*d*) neutrophile tissues remain indifferent both to acid and to basic stains. A successful technique should enable us to recognize and to classify tissues according to these varieties. Hence, the staining characteristics of cell tissues are best revealed when we employ an acid and basic stain, or what is known as double staining. This may be accomplished by two distinct methods: By using the stains (*a*) in conjunction, or (*b*) in succession. By the first method both stains are combined in the same solution. The second method requires two distinct solutions, the one following the other. In many regards I find the latter more desirable as well as more expedient.

In making a choice of dyes, two points should be considered: (*a*) There should be a wide contrast in color, and (*b*) the stains should act quickly. In these respects eosin is the ideal acid dye. And methylene blue, making a beautiful contrast, is one of our most reliable and durable basic dyes. I have observed that staining first in a slightly acidulated eosin solution, and counterstaining in a methylene blue solution rendered alkaline, not only bring out the granules of the cell body more distinctly, by giving them a brighter tint, but increase the contrast between the oxyphile and basophile tissues of the cell. My rule, therefore, is to use an acid stain in an acid bath, and a basic stain in an alkaline bath. Hence, I use the two stains prepared according to the following formulæ:

No. 1.

Spirituous eosin (Grübler's)	0.25 gm.
Alcohol absolute (Squibb's)	35 cc.
Distilled water	15 cc.
Aqueous solution of acetic acid (glacial) 1 per cent.	10 minims.

Mix and allow to stand in an open vessel until, by evaporation, the quantity is reduced to 35 cc.

No. 2.

Methylene blue (Grübler's)	0.30 gm.
Alcohol absolute (Squibb's)	15 cc.
Distilled water	50 cc.
Caustic potash, C. P.	10 mgm.

Mix and allow to stand in a closed bottle for one month before using.

Precaution should be taken not to overstain in either of these solutions. The length of time which gives best results will depend upon the quality of the ingredients, the age of the solution, the amount of evaporation, and the quantity of acid and alkali added to these solutions. When the proportions are properly adjusted for each solution, excellent results can be secured by staining in the eosin fifteen seconds and counterstaining in the methylene blue ten seconds.

It is important to note that there are two varieties of eosin used in laboratory work: (*a*) aqueous and (*b*) spirituous.

(*a*) Aqueous eosin in aqueous solution is more or less removed from the tissues in the process of washing. I have observed that by adding alcohol to this solution the eosin is more permanently fixed in the tissues, and also that it is not so readily removed by washing. In this form aqueous eosin stains granules very distinctly. But hæmoglobin is stained less rapidly and less deeply, while it is quickly decolorized and counterstained by

the basic stain. On the other hand, the granules are not so readily decolorized, but remain beautiful and clearly defined.

(*b*) Spirituous eosin stains hæmoglobin quickly and deeply, and is not readily decolorized by the basic counterstain. It also stains the granules. But if overstaining is permitted, granules are so deeply and extensively stained that they seem to blend together, and their clearly defined contour is lost.

Hence, I have found that aqueous eosin in a diluted alcoholic solution, very slightly acidulated, followed by the basic counterstain, is quite satisfactory in bringing out the garnules and in showing the structures of nuclei. But overstaining in the basic counterstain should be avoided.

Spirituous eosin will show the red cells to a far better advantage, but it must be more strongly acidulated. Freshly prepared staining solutions are often unsatisfactory. Hence, it is never safe to compare a recently prepared staining solution with one that has been in use for some time, unless they have first been tested on films of the same blood. Hence, to secure comparative results I always (*a*) use the same strength of staining solution; (*b*) stain a uniform length of time, and (*c*) stain the shortest period that will give satisfactory results. When these points are observed and uniform results are not obtained, we may be quite certain that the failure depends upon a variation in the condition of the blood, and not upon a variation in the technique.

(*D*) Washing and Mounting: After staining the films, they are washed in a normal salt solution (0.6 per cent. aqueous solution of sodium chloride), by slowly passing the cover glass through the solution two or three times. Excessive washing should be avoided. The films are then dried between sheets of fresh blotting paper. After staining in the eosin and drying the film, the cover glass is quickly passed through an alcohol flame two or three times, the film surface uppermost. This is done to fix the eosin. Overheating should be avoided. After the staining is completed, the specimen is permanently mounted in Canada balsam. The mounted specimen is then ready for study.

(*E*) System of Study: If a systematic method of classifying and recording the phenomena is not adopted, one becomes bewildered with the material before him. I

find it a great help to observe the following points: (*a*) A mechanical stage should always be used; (*b*) also the same ocular and lens to secure uniform magnification; (*c*) a high power is very necessary; (*d*) good fields for observation should be selected; (*e*) we should avoid excessive or insufficient illumination, and should not study by artificial light; (*f*) and finally, a large number of observations should be made, and, if in doubt, a second film should be obtained and studied before making deductions. If the delicate changes which take place in the process of cell disintegration are to be utilized for diagnostic purposes, too much precaution in these points can not be taken.

INTERPRETATION OF PHENOMENA.

Before studying the appearance of cell tissues in disease, it is first necessary to fix in one's mind the shade or tint that a staining solution communicates to cell tissues in the normal state. This can be done only by long and persistent study. It then becomes comparatively easy to recognize abnormal conditions by a variation in the staining reaction of these tissues.

THE PHYSIOLOGICAL CHEMISTRY OF CELLS.

Believing that something more definite could be ascertained concerning the physiological chemistry of cell tissues and their nature and conditions, I recently undertook a series of staining experiments. I will briefly report the result. For each experiment six blood films were uniformly prepared and fixed. They were then stained, according to the methods to be described, mounted, numbered, and the technique recorded for each film. The following are the principles embraced in the experiments made:

First Experiment—Films stained for varying periods of time in an acid stain (five seconds to five minutes).

Second Experiment—Films stained for varying periods of time in a basic stain (five seconds to five minutes).

Third Experiment—Films stained in an acid stain for a uniform period of time (fifteen seconds), followed by counterstaining in a basic stain for varying periods of time (five seconds to five minutes).

Fourth Experiment—Films stained in a basic stain for varying periods of time (five seconds to five minutes), followed by counterstaining in an acid stain for a uniform period of time (fifteen seconds).

The firms thus prepared revealed a great variety of phenomena. At first these seemed perplexing, but subsequent study revealed laws by which the phenomena could be classified and interpreted.

Experiment I.—Effects of an acid stain on cell tissues:

(*a*) Red cells stained quickly and deeply. Hence, hæmoglobin is distinctly oxyphile.

(*b*) All nuclei remain transparent. Hence, they are not oxyphile.

(*c*) All cell bodies react more or less to the acid stain. Hence, they are more or less oxyphile. The oxyphile tissue of the cell body appears as granules. This is probably hyaloplasm. The granules are observed over the entire cell body. Those appearing over the nucleus are not within the nucleus, but within that part of the cell body which intervenes between the nucleus and the observer.

That part of the cell body of young cells immediately external to the nucleus appears as a distinct ring or zone, which is strongly oxyphile. This zone fades or becomes less oxyphile toward the periphery of the cell. Hence, the contour of these cells is not clearly defined when stained by an acid stain alone. Therefore, the oxyphile quality of the cell body of young cells is greatest near the nucleus and decreases toward the periphery of the cell. If hyaloplasm is the oxyphile tissue of the cell body, it would then appear that hyaloplasm predominates in that portion of the cell body of young cells nearest the nucleus and decreases toward the periphery of the cell.

The cell body of phagocytes or mature leucocytes is not only more distinctly oxyphile, but is uniformly oxyphile throughout.

The cell body of eosinophiles is strongly oxyphile and uniform throughout.

Therefore, it appears that as the cell develops and approaches maturity, the oxyphile quality of the cell body increases. Hence, the minimum proportion of hyaloplasm is found in young cells, and the maximum proportion is found in the older or mature cells. I have also observed that, as cell disintegration advances, the oxyphile quality of the cell body decreases.

Experiment II.—Effects of a basic stain on cell tissues:

(*a*) Red cells stain a light green, which becomes darker the longer the film remains in the staining fluid. Hence, hæmoglobin is also basophile. From our first experiment it was found to be oxyphile. It is, therefore, both oxyphile and basophile, or an amphophile substance.

(*b*) Nuclei react to a basic stain. Hence, they are distinctly basophile. They are never oxyphile. They may, however, remain almost indifferent to a basic stain. There is, then, a deficiency in chromatin, or the network of the nucleus, which bears the same relation to the nucleus as spongioplasm bears to the cell body.

Chromatin possesses the basophile quality of nuclei. In normal nuclei this is usually well marked in young cells, less marked in phagocytes, and still less marked in eosinophiles. Hence, it seems that the basophile quality of nuclei decreases as they mature. The age of the cell is, therefore, an important consideration in estimating the staining quality of the nuclei. Achromatin is a neutrophile substance, and bears the same relation to the nucleus as hyaloplasm bears to the cell body.

(*c*) All cell bodies react more or less to the basic stain. Hence, they are more or less basophile. The basophile tissue of the cell body appears as a network. This is probably spongioplasm. Transparent or opaque granules are observed over or within the basophile network of the cell body of all varieties of leucocytes. These granules, therefore, are not basophile. In young cells these granules are very indistinct, but become more distinct as the cell develops. That part of the cell body of young cells immediately external to the nucleus often appears as a distinct transparent ring or zone, while the external portion of the cell body is well stained. Hence, the contour of these cells is clearly defined when stained by a basic stain alone. Therefore, the basophile quality of the cell body of young cells is least near the nucleus, and increases toward the periphery of the cell. If spongioplasm is the basophile tissue of the cell body, it would then appear that spongioplasm predominates in that portion of the cell body of young cells nearest the periphery of the cell and decreases toward the nucleus.

The cell body of phagocytes or mature cells is not only less distinctly basophile, but is uniformly so throughout.

The cell body of eosinophiles is very faintly basophile, and is uniformly so throughout.

Therefore, since the basophile quality of the cell body is well marked in young cells, less marked in phagocytes or mature cells, and still less marked in the eosinophiles, this experiment seems to justify the conclusion that the basophile quality of the cell body decreases as the cell develops and approaches maturity. Hence, we conclude that the maximum proportion of spongioplasm is found in young cells and a minimum proportion is found in the older or more mature cells. According to this law, it appears that the eosinophile cell is a more highly differentiated cell, or a cell of higher development. On the other hand, I have observed when cell disintegration begins that the reverse of this law takes place, and the basophile quality of the disintegrating tissues increases. This is also observed in the disintegration of nuclei. As disintegration advances, the basophile quality of adult nuclei increases—as observed in nuclei of pus cells, which are very strongly basophile. Hence, if these observations have been correctly made, we may formulate two laws:

THE LAW OF DEVELOPMENT.

As cell development progresses, the oxyphile tissues of the cell become more oxyphile, and the basophile tissues become less basophile.

THE LAW OF DISINTEGRATION.

As cell disintegration progresses, the oxyphile tissues of the cell become less oxyphile, and the basophile tissues become more basophile.

Experiment III.—Effects of staining with an acid stain and counterstaining with a basic stain:

(*a*) Red cells, after being stained in an acid stain, become a deeper oxyphile when exposed to a basic stain for a short time. When the counterstaining is prolonged, the oxyphile tint decreases and is finally lost, while the basophile tint increases until the cells present a deep purple tint. Hence, hæmoglobin reacts to both an acid and a basic stain. It is, therefore, an amphophile substance, but shows a much stronger affinity for an acid than for a basic stain, and reacts to it more quickly.

(*b*) Nuclei present the same appearance as in our second experiment; that is, they appear distinctly basophile.

(*c*) The cell body of young cells appears distinctly basophile, with no evidence of an oxyphile tint. Hence, the faintly granular hyaloplasm of these cells, which took a distinctly oxyphile tint in our first experiment, is neutralized or decolorized from the action of the basic counterstain, and appears transparent, even when the counterstaining is for a short period of time. These transparent granules are set in a deep basophile background.

The cell body of phagocytes appears distinctly oxyphyle. The oxyphile quality is confined to the granules, which seem to be set in a faintly basophile background. Hence, both the spongioplasm and the hyaloplasm are stained, the one being faintly basophile and the other distinctly oxyphile. The effect of the basophile tint of the spongioplasm, with its contrast in color, tends to bring out the oxyphile tint of the granules even more distinctly.

PROLONGED COUNTERSTAINING.

Prolonged counterstaining with the basic stain decreases the oxyphile tint of the granules of phagocytes until it is finally lost. But the granules at no time show a basophile tint. While, on the other hand, the basophile tint of spongioplasm gradually deepens up to a certain point, it then ceases to change, the ultimate tint varying according to the age or condition of the cell.

Hence, it is evidence of disintegration if mature oxyphile tissues, stained in an acid stain, are quickly decolorized when counterstained in a basic stain, or when basophile tissues show an unusual susceptibility to the basic stain. Prolonged counterstaining has much less effect on the granules of the eosinophile cells, than upon the granules of phagocytes. Hence, they retain their oxyphile tint much longer; at the same time the spongioplasm is less distinctly basophile.

Therefore, it appears that the granules of eosinophile cells are strongly oxyphile, while the granules of phagocytes are oxyphile to a less degree, and the granules of young cells are faintly oxyphile. Hence, it seems that all granules are distinctly oxyphile, but that their affinity for the acid stain increases as the cell develops and matures.

In a former paper I made the statement that granules are first basophile; that they become oxyphile; and, if the cell lives long enough, they again become basophile. The foregoing experiments are more recent, and demonstrate that my first interpretation was probably erroneous.

The granules of all leucocytes, even the youngest, according to our first experiment, are shown to be distinctly oxyphile. The second experiment shows that they are not basophile. And the third experiment demonstrates that in young cells granules are quickly decolorized by the basic counterstain, while in more mature cells they remain distinctly oxyphile, and by prolonging the basic counterstaining they finally lose their oxyphile tint. Hence, it seems that we may be justified in making the following deductions:

(*a*) Granules of the cell body of young cells are faintly oxyphile. (*b*) The oxyphile quality increases as the cell develops. (*c*) When disintegration begins, the oxyphile quality decreases as the cell disintegrates. Further research is needed in this line before dogmatic statements should be made. It demonstrates one point, however, that uniform technique is absolutely essential if we wish accurate results.

DEDUCTION.

A basic stain decolorizes oxyphile tissues very slowly. Hence, it is an ideal counterstain.

RULE FOR BASIC COUNTERSTAINING.

The time allowed for a basic counterstain should be extended to the point that basophile tissues are well stained without decolorizing the mature oxyphile tissues.

Experiment IV.—Effects of staining with a basic stain and counterstaining with an acid stain:

(*a*) The basophile tint transmitted to the red cells by the basic stain (as shown in Experiment II.) is quickly decolorized by the acid counterstain, and the cells are quickly restained by the acid stain.

(*b*) The basophile tint of nuclei is also quickly decolorized, and they remain transparent. No amount of overstaining will give them an oxyphile tint.

(c) Spongioplasm of all cells is decolorized and remains transparent.

DEDUCTION.

An acid stain quickly decolorizes all basophile tissues. Hence, it is not a satisfactory counterstain.

THE LAW OF ANALOGY.

A study of the larger animal organism in health reveals the fact that some of the body fluids are acid, while others are alkaline. In diseases these reactions are often altered or reversed. Hence, in the larger animal organism, when the functions are changed, the reactions of the secretions and excretions are altered. Or, inversely, when the reactions of the secretions and excretions are altered or reversed, it is evidence of perverted functions.

It will suffice to speak of only a few of the changes that occur in the chemical reaction of the body fluids in disease. The reaction of the saliva in health is distinctly alkaline. In certain diseases it may become acid. The normal reaction of the gastric juice is acid. This may increase until it becomes what is known as hyperacidity, or it may decrease until the acid reaction is absent. These extreme reactions are well-known points of the highest clinical significance. On the other hand, there can be but little doubt that equally as great changes take place in the chemical and staining reaction of cell tissues as are known to take place in the tissues and fluids of the larger organism.

The foregoing experiments have shown conclusively that the tissues and fluids of leucocytes have complex staining reactions, and that these reactions change with the growth and decay of the cells.

Histologically and chemically we find leucocytes to be extremely complex organisms. Between the nucleus and cell body there is a distinct dividing membrane. This membrane separates tissues of quite distinct staining reactions. On its inner surface it is in contact with two substances—the one neutrophile and the other basophile. On its outer surface it is in contact with two substances—the one distinctly oxyphile and the other basophile. The leucocyte itself is floating in the blood serum, which holds in solution the elements of nutrition, mingled with toxins and the complex products of cell

and tissue disintegration. Through the membrane which separates the nucleus from the cell body, osmosis plays an important part in the vital process of the cell. Whatever nutrition the nucleus receives must pass through this membrane by the process of endosmosis. Whatever secretions or excretions are eliminated from the nucleus must pass through this membrane by the process of exosmosis. Hence, there are delicate chemical processes taking place in the cell, equal to any going on in the larger body. Therefore, it is evident that as we descend the scale of life, we find that the same laws are in force. The intricate chemical processes which are continually taking place in the larger organisms are at work in its cells. Therefore, by analogy, if the staining reaction of any cell is observed to vary from that found in normal cells, we may conclude that there must be a fundamental cause within the cell. And since a change of appearance in tissues is preceded by a change in function, we may conclude that the cause of this unusual susceptibility to stain is a perverted function of the cell. This gives us another law of analogy between the larger animal organism and its leucocytes.

In conclusion, I wish to make a brief reference to the application of these observations to

THE DIAGNOSIS OF TUBERCULOSIS.

A careful study of one hundred tuberculous cases, including all stages of the disease, has shown conclusively that the law that brings about disintegration in a tuberculous patient brings about the same process at an earlier date in tuberculous leucocytes. In these cases I have found (*a*) marked disintegration going on in all varieties of leucocytes, and (*b*) a great decrease in the number of young cells. These conditions are evidence that the tissue-forming power of these cells is imperfect or defective. Hence, when these conditions exist in cells, we may be absolutely certain that a similar condition already exists, or soon will exist in the larger organism. Therefore, from the condition of the various cell tissues observed in these cases, I feel justified in making the following

DEDUCTIONS.

First, it is possible to estimate the degree of the tuberculous condition. Second, it is possible to estimate the degree of the recuperative power. Furthermore, the

phenomena upon which the foregoing deductions are based are as follows: (1) The degree of the tuberculous condition may be estimated by (*a*) the amount of deviation from the normal percentage of each variety of cells; and by (*b*) the amount of cell disintegration in each variety. (2) The degree of the recuperative power may be estimated by (*a*) the staining power of the nuclei; (*b*) the percentage of leucocytes with no evidence of disintegration; (*c*) a relatively high percentage of young cells; (*d*) the abundance of well-stained granules of the phagocytes; and (*e*) the abundance of eosinophile cells rich in granules. I do not wish to give the impression that it is an easy task to interpret the phenomena presented in a specimen of tuberculous blood, and from them to reach a diagnosis. But it can be done. And when it is properly done, it furnishes a diagnosis based upon the fundamental principles of biology. Hence, from a study of the foregoing cases, I feel justified in claiming that the blood, aided by the microscope, together with a uniform and accurate technique, furnishes a means of making a positive diagnosis of the tuberculous condition early enough to allow of effective treatment.

205-206 Jacobson Block.