THE VALUE OF BLOOD EXAMINATION IN THE DIAGNOSIS OF DISEASE.

GRADUATING THESIS.

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Physiologically the blood may be considered simply as a medium of exchange, collecting the tissue forming elements from the lungs and the assimilated products of the digestion of the food, distributing these to the various tissues of the body there to be made use of in the anabolic changes, and in turn collecting the products of catabolism to carry these to the liver and the excretory organs -- skin, kidneys, lungs and possibly the bowels.

Histologically it may be considered as a tissue composed of colorless blood cells and red blood cells, floating in a liquid, the blood plasma. The colorless cells originate in lymphoid tissue and in bone marrow. There are several varieties of colorless cells recognized, which includes the following:

1. Small lymphocytes, about the same size as the red cell and containing a relatively large nucleus. This variety probably originates wholly within lymphoid tissue.
2. Large mononucleur leucocytes, three times as large as the red cells and containing a nucleus.
3. Transitional leucocytes representing a more advanced stage of development, and containing a nucleus often kidney shaped or indented.
4. Polynucleur leucocytes. This is the form found most abundand and represents the fully developed leucocyte. This va-

riety is recognized by the distorted nucleus. The segments usually retain their entirety, the segments being joined by chromatin, but often these segments become separated, giving rise to the term polynuclear.

The white blood cells bear a very important relation to disease, and this phase of their physiological functions and morbid processes will be treated later.

The colored blood cells are disc shaped and in the human blood, measure about 1/3200 of an inch in diameter. They contain no nucleus. About 90% of the red cells is made up of hemoglobin, a complex albuminoid substance which forms a loose compound with oxygen. The red cells thus absorb oxygen from the air of the lungs during the passage of the blood through that organ, and this is carried to the tissues where it is utilized in the oxidation of the carbon, the carbon-dioxide thus formed, being absorbed by the hemoglobin and in turn given off to the lungs.

The life of a red blood cell is probably about three weeks, and they are formed in the red marrow of bones, the marrow of the ribs probably being their most important source. They are broken down in the liver and spleen. The number of red cells present in a few of the various animals per cubic M.M., is approximately as follows:

Man -- 4,500,000.

Cow -- 6,152,000.

Dog -- 4,500,000.

The number varies somewhat with age, sex and condition of health. Disease may greatly decrease the number of red cells, but aside from that, they bear no very important relation to disease except in so far as they assist in metabolism.

The blood plasma is the liquid in which the solid constituents are transported. It includes about 65% of the blood, and is composed of about 90% water and 10% solids. Of these solids about 8% are proteid in nature, and the remaining 2% is made up of inorganic salts and a class of substances known as extractives. The solids of the blood plasma may be profoundly changed by disease, and this phase will be treated later.

The Pathology of the Blood.

The blood may be the means of transporting poisons from the place of their entrance into the body, to the organ or organs for which the poisons have a special affinity, or the poison may act directly upon the blood itself.

Poisons may be divided according to their origin, into mineral poisons, vegetable poisons, and animal poisons. In this article we are concerned especially with the vegetable poisons. These may be again divided into the vegetable alkaloids and those produced by the lower forms of vegetable life and include toxic cadaveric alkaloids, toxic ptomains, toxins, toxalbumins, and toxenzyms. Probably most all of the more profound disturbances of the bodily health is caused by some of these forms of poisoning. Again we may divide these poisons into those formed withing the body following the introduction into the body of their producing agent. The poison may thus produce local tissue change and disease, or by being transported by the blood, may produce general poisoning of the organism, or through metastatic changes, may affect some particular organ or set of organs; or this organism which produces the toxic substances may be transported in the blood stream to other parts of the body, when it may set up a new seat of infection.

The poison which probably the most often produces disease changes

in the body, is the toxalbumin, and is produced from the bacteria cell itself. It differs from the toxins in that it produces the symptoms of poisoning much slower than the toxins. The toxalbumin may be neutralized in the blood serum by proper methods, and this fact has an important bearing upon the treatment of disease, as will be noticed later.

These toxalbumins may affect the organism in several ways, the most important of which is their effect upon the sympathetic nervous system, disturbing thermogenesis and thermolysis, thus causing the body temperature to become abnormal. They may cause increased blood pressure and acceleration of the pulse, but this phenomonon is generally considered as a prophylactic agent to assist in eliminating the poisonous agents from the body and in attempting to overcome their injurious effects. T he bacteria themselves or the toxalbumins formed by the bacteria, may play an important role in the destruction of the red blood cells, and in this connection as well as in the toxalbumin formed and the disturbances of the infected organism, may be included the trypanasoma. The influences these organismsand albuminous bodies may thus have on the metabolism of the body, may be readily seen.

Natural Immunity.

Nature has provided agents antagonistic to the entrance into the body and the development of the organisms within the body, which may produce the poisonous substances. In this connection we may consider the natural immunity of the body or the resistance which the body displays to the entrance into the system of infecting agents. First we may take the external covering of the body, the skin, which is very resistant to the entrance of most pathogenic organisms, probably because of its being a poor culture media for such organisms, and be-

cause of its comparative isolation from the blood which might convey the organisms to more favorable seats of infection. Only when the skin is broken as in wounds, does it present an important source of infection. Probably the most important source of infection is the mucous membranes of the respiratory and digestive tracts, and when we consider the apparently favorable conditions for development of organisms such as darkness, moisture, and heat, and the permeability of the mucous membrane, we must recognize some antagonistic agent to counteract the likelyhood of this source of infection. It may be a chemically active substance secreted by the mucous membrane which may be antagonistic to infecting organisms, in various degrees as to entirely hinder the entrance of the organism into the body, as for instance swine plague and symptomatic anthrax in the human.

The agents hindering the entrance into or the spread of infection after entrance into the body, may be divided into those forces extrinsic to the body, as the movement of cilia in the respiratory tract, which tends to remove the organisms from the body,and the poisonous action of the gastric juice and the bile to many forms of pathogenic organisms. The mucous secretions also play an important part in antagonizing the development of an entrance into the body of bacteria.

When organisms succeed in gaining an entrance into the body, but have not yet entered the blood stream, there are agents present which may antagonize the further development of the organism, or neutralize the poisonous products formed. The most important phase of this process is that which is commonly designated as phagocytosis. It consists primarily in the envelopment of the invading organism by the leucocytes, and may occur within the blood stream or outside of it in the lymph spaces. This process is considered as one of the

vital processes of the leucocytes. When an infecting organism gains entrance into the body and begins to develop and multiply, a gathering of the leucocytes at that place may be noticed. The reason assigned to this is the attraction certain substances have, or the stimulating action certain substances may have on certain tissues, the invading organisms acting as a stimulant for the leucocytes, this being commonly referred to as chemotaxis. The leucocytes by an ameboid movement, envelop the organism and if they are not present in too great numbers, or if their resistance is not too great, they may be entirely absorbed by the leucocytes and removed from the body, or the leucocytes may themselves be destroyed and the invading organism dominate. Another agent which inhibits the multiplication of invading organisms, is a chemicaly active albuminoid substance which attacks the organisms after their entrance into the blood stream. This substance may be present normaly in the body and thus prevent the entrance of the organisms, or it may be produced during the course of an infection. In support of this view we have the weakening of the virulence of the organisms after the disease has progressed to a certain stage, and finally the death of the infection.

We find all of these protective agencies present in a more marked degree in some individuals than in others, and in the same individual at certain times or under certain conditions they are more pronounced than at other times or under other conditions. They are also more active toward certain organisms than they are toward others. Thus we have a difference in degree of immunity in different individuals and in the same individuals under different circumstances.

Acquired Immunity.

The body may acquire immunity against invading disease in several ways. First we may have immunity acquired through a previous

attack of the disease. It is a fact of common observation, that an attack of many diseases produces an immunity against a subsequent attack of that disease.

Second, we may have immunity produced by inoculation with an attenuated culture of the organism of a disease. In this way we may have the disease produced in such a mild form or so localyzed as to be insignificant , yet producing an immunity against an attack of the disease.

Another form of acquired immunity is produced by the injection into the patient of blood serum taken from an animal immune to the disease through a previous attack or through inoculation. This form of protection is made use of even after infection has taken place, and it, like the two preceeding modes of immunization, exerts its power through the production in, or injection into, the system of a counter poison or anti-toxin which acts antagonistically to the toxin produced by the invading disease.

Since the blood plays such an important part in its relation to disease, it would seem but natural that an examination of the blood would often reveal the true nature of the disease, and with a great many diseases we find such to be the case. Hematology as a means of diagnosing disease in the human patient, has been developed and employed to a considerable extent. With most all the more important diseases, the condition of the blood has been studied until it has been definitely determined the condition of the blood in most of these diseases. The data afforded by a careful examination of the blood, while not pathogenic, is nevertheless valuable in giving a correct diagnosis.

In veterinary medicine, however, very little work has been done along this line, and judging from the value ascribed to this work in

the human practice, there is a broad field for investigation for the investigator who wishes to do original work along this line. It is doubtful, however, if the same value will ever be ascribed to this work in the veterinary practice which it receives in human medicine. The reasons for this are many, among which may be mentioned the unsatisfactory conditions under which the samples must be taken. In making a bacteriological examination of the blood, it is very much more difficult to control the conditions so that the sample will be aseptic, than is the case with the human patient. Also, in taking samples to determine the number of red and white cells present, a degree of care in the technique of the operation is necessary, which is incompatible with any movement on the part of the patient, and the irritation incident to taking the sample, only tends to increase the animal's restlessness.

Considerable work has been done, however, by Smith & Kilbourn in their investigation of the etiology of Texas fever. Several investigatiors have done considerable histological work along this line, and Drs. Dimock and Thompson have done a limited amount of histological and pathological work.

The characteristics of the blood which are determined and studied for the value they may have in aiding in the diagnosis of the disease, are --

1. Microscopical examination of the fresh blood.
2. Estimation of the percentage of hemoglobin.
3. Counting the enthocytes and the leucocytes.
4. Microscopical examination of stained specimen.
5. Determination of the serum reaction or the agglutination test.

In making a microscopical examination of the fresh specimen, the

field of operation is shaved and disinfected thoroughly. In domestic animals the under side of the tail a few inches from the body, presents the best field. A deep, clean incision should be made, preferably with a spring fleam. The first drop of blood should be wiped off and the next drop should be touched with the edge of a rectangular cover slip, chemically clean. A second cover slip is then laid on top of the first one, and then carefully removed by drawing it away parallel to their flat surfaces. This should cause an even thin spread of blood over the entire glass. The slides may be examined immediately, or allowed to dry. The examination should be made with the $\frac{1}{6}$ objective. In this examination the shape of the red cells may be noted, whether they appear to be disintegrated and whether there are any microorganisms present or not.

The technique is very simple and it presents a method of securing valuable evidence in regard to the true nature of the disease, and this examination should form the initial step in any examination of the blood.

The estimation of the hemoglobin is a somewhat complicated proceedure, and is of only minor clinical importance in the majority of cases. The instrument in most common use, consists of a tinted glass wedge. A small stage on the microscope which can be moved across the field, a mixing chamber, a capillary pipette, and a fine pointed glass dropper. The value of this test is primarily to determine the oxygen carrying power of the blood..

In estimating the red and white cells, the selection and preparation of the field is the same as for making the microscopical examination. It is necessary that the blood be greatly diluted to facilitate the counting, and for this purpose several special diluting fluids are employed. Probably the simplest dilutant for the red

cells is a 3% sodium chloride solution. The instruments necessary for counting the red cells are a Thoma-Zeiss Hemocytrometer, which consists of a heavy glass capillary tube the lumen of which is expanded near the upper end. The capillary tube is graduated and marked .5 and 1; just above the dilated portion is the mark 101. The counting chamber consists of a heavy glass slide in the center of which is cemented a square glass plate provided with a circular opening which fits around a disc which is ruled into squares each one of which has an area of $\frac{1}{400}$ sq. M. M. This disc is exactly $\frac{1}{10}$ M. M. below the square glass plate, the cubic contents of each square being thus $\frac{1}{4000}$ c. M. M.

In making the count, the field of operation is thoroughly washed and disinfected and a smooth, free incision is made through the skin, facia, and into the muscles, so that a free flow of blood is secured. The incision must not be pressed, as this presses the lymph out with the blood, thus diluting it and destroying the accuracy of the count. The first drop of blood is wiped away, and the capillary pipette is touched to the next drop that starts, and the blood is drawn up exactly to the mark. The greatest care must be taken to prevent the entrance of air into the tube, and should this occur, the pipette must be cleansed and dried, and the operation repeated. As soon as the blood is drawn into the pipette, the point is wiped off and inserted in in the 3% sodium chloride solution, and filled to the mark 101, and this is then thoroughly mixed. A few drops are then blown out, and a small drop is placed on the center of the counting chamber and a clean cover slip is then pressed down.over the counting chamber. The drop should thus be made to spread exactly to the edge of thefurrow surrounding the counting chamber. The specimen is then placed in a microscope and adjusted. A microscope

with a $\frac{1}{5}$ objective, with a mechanical stage and with the condensor removed, is preferable. The red cells may now be seen as disc shaped bodies with numerous short, sharp projections. They should be counted carefully, moving the microscope along one side of the ruled space to the edge and returning on the next tier of wquares until fifteen or twenty squares have been counted. A regular order should be followed in order to obviate the danger of recounting the same erythrocytes; an average is thus secured of the cells in each square. This represents the number of cells found in each $\frac{1}{4000}$ c M. M. Since the sample was diluted with 100 volumes, the total number of erythrocytes per c M. M. is equal to the average in each square multi;lied by 4000 times 100.

It is needless to say that the greatest care is necessary in taking the sample and making the dilution, and the pipette and counting chamber must be kept strictly clean.

In counting the leucocytes the technique of the operation is the same. The instruments used are the same except the pipette, which is graduated .5 1, and above the bulb 11. The diluting fluid in this case should be a $\frac{1}{3}$ of one percent acetic acid solution. A few drops of Gentian violet may be added to this to stain the leucocytes. The dilution now, instead of being 1 to 100 is 1 to 10. The process is otherwise the same as for the red cells. The number of leucocytes per c. M. M. averages about 8000 for adults.

The preparation of the stained specimen is practically the same as for the microscopic examination of the past specimen, except that the specimen is thoroughly dried and set, and then stained. This serves to bring out more definitely the elements and also any foreigh matter present.

Widall's reaction or the agglutination method of diagnosing

disease was developed first by Widal in 1896 as a means of diagnosing typhoid fever. The technique of the operation consists in the addition to a bouillon culture of bacili typhus of a few drios if fresh blood from the supposed typhoid patient. In a few minutes the characteristic cloudiness of the culture disappears and the sediment settles to the bottom. A modification of this reaction, which is now most commonly used, is to use only a drop of blood on a cover slip and the addition of a few drops of bouillon culture directly to this to secure the proper dilution, and examining the slide under the microscope immediately to observe the agglutination. A modification of this method is now employed quite extensively and with good success, in diagnosing glanders. This method is employed as the official method of recognizing the disease in Germany. It has the virtue of being comparatively certain, though somewhat more difficult to handle than is the Mallein test.

Summary.

Many disease processes are manifest directly on the blood, and many are carried over the body by means of the blood.

Since the blood bears such an important relation to disease, it is but natural that an examination of the blood would give valuable evidence in regard to the etiology of disease processes.

The blood is also important because of its relation to therapeutic treatment of disease.

Hematology has assumed a place in human medicine of considerable importance; the work along this line in veterinary medicine is somewhat limited. While this line of work will probably grow in importance in veterinary medicine in discovering the etiology of disease and thereby the rational therapeutics of the disease, yet from the

nature of the work, this will be left to the investigator. The conditions under which the practitioner is compelled to work precluding the probability of his doing very much work along this line.