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The Nature of the Leucocytosis Produced by  
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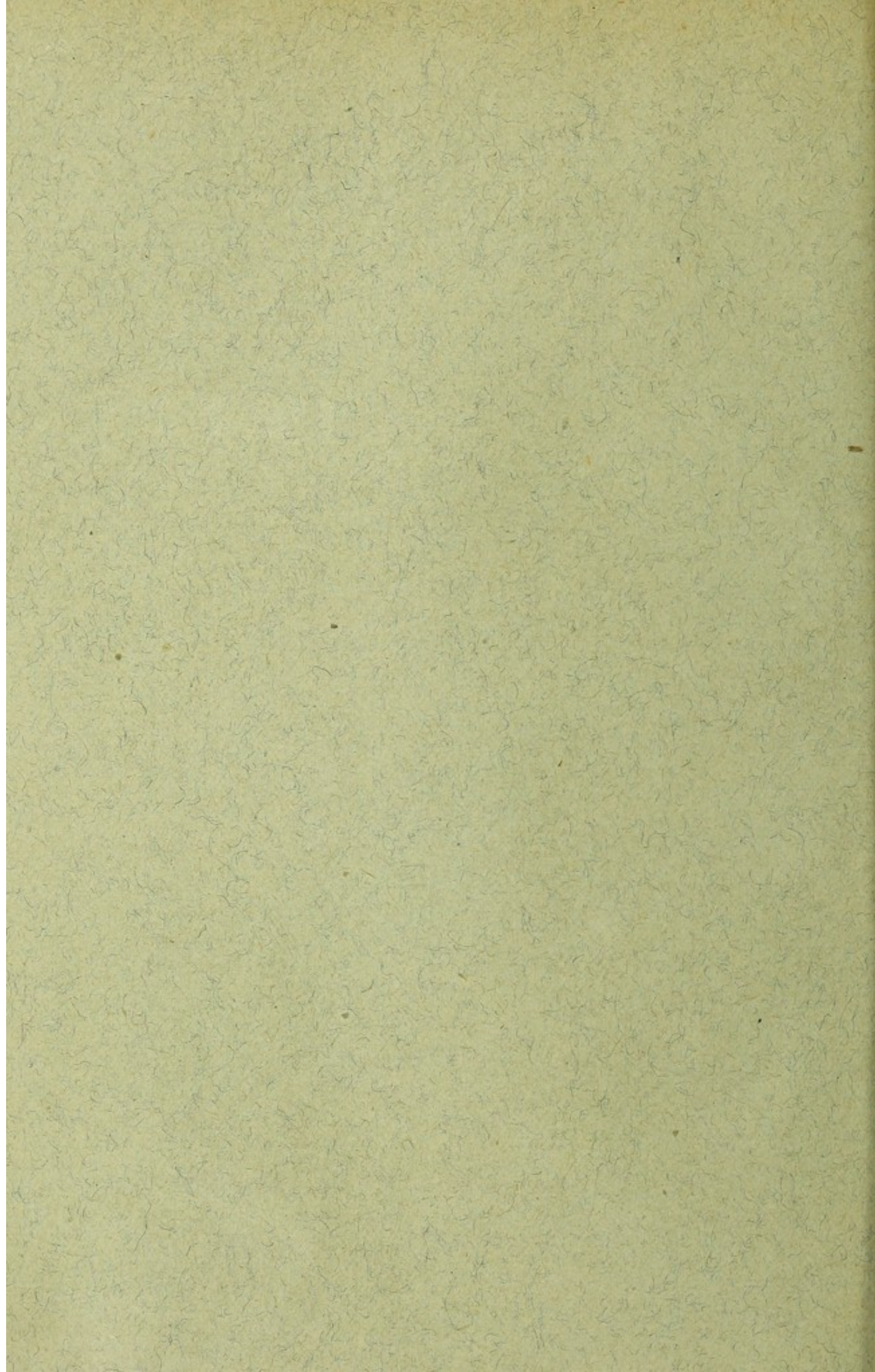
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THE NATURE OF THE LEUCOCYTOSIS  
PRODUCED BY NUCLEINIC ACID;  
A PRELIMINARY EXPERIMEN-  
TAL STUDY.

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AND A. A. HUNTLEY, M.D.

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Within the past two or three years there has come into gradual prominence a system of therapeutics known popularly as nuclein therapy, which is attracting more and more attention and receiving a growing recognition at the hands of the medical profession.

The use of nuclein was first advocated on purely theoretic grounds, it being claimed that by its use the germicidal power of the blood could be increased and hence the resistance to diseases of germ origin heightened. Of late, however, certain writers have denied that nuclein acts as it was supposed to, and in consequence, its use being founded on a false conception of its powers, have claimed that it was not beneficial.

The application of nuclein to the treatment of certain diseases, especially to certain of the infectious processes, seems to be attended with beneficial results, though the observations are as yet too few and too incomplete to be of much value. Such as they are, however, they tend to confirm the views on which its use was first advocated and should be taken for what they are worth.

In order to fully comprehend the point at issue, it will be well to briefly review the various steps that have led to the discovery and use of this interesting substance.

Ever since the announcement of the fact that nor-



mal blood plasma of man and animals possessed definite germicidal power, endeavors have been made from time to time to determine the nature of the substance that imparted this property to the blood, in the reasonable hope that with its discovery we would be put in possession of the means to artificially increase this bactericidal property at will, which it is readily conceded would be of considerable importance in the treatment of certain diseases.

As early as 1872, the fact that organisms when injected into the circulation rapidly disappeared was observed by Lewis and Cunningham.<sup>1</sup> Similar facts were recorded from time to time by a number of observers, among whom may be mentioned Traube,<sup>2</sup> Fodor,<sup>3</sup> Wysokowicz,<sup>4</sup> Grohmann,<sup>5</sup> Nuttall<sup>6</sup> and others, and numerous theories, which it would lead us too far to mention or discuss at this time, were advanced to account for the apparent destruction of the invading organisms on the part of the body.

No explanation not open to serious objection was offered until the publication of the work done by Nuttall under Flüge. He claimed that the injected organisms disappeared because the normal plasma was germicidal and destroyed them. He gave positive evidence of this fact.

Following the work done by Nuttall, Nissen,<sup>7</sup> also working under Flüge on the same question, came to the following conclusions among others: 1, that normal blood plasma can easily destroy the cholera germ and Eberth's bacillus; 2, that the addition of small quantities of sterilized bouillon or of normal salt solution would not destroy the germicidal property of the blood; 3, that for a given volume of blood there is a maximum number of bacteria that can be destroyed; 4, that blood rendered uncoagulable by the addition of peptone is still germicidal; 5, that blood the coagulation of which is prevented by the addition of 25 per cent. of magnesium sulphate has its germicidal property diminished; 6, that filtered blood plasma from the horse is germicidal.



While, then, it was clearly proved that normal blood possessed certain properties detrimental to the growth and development of bacterial life, it was not as yet shown to what this property was to be attributed. Among some of the ideas advanced may be mentioned that of Behring,<sup>8</sup> who believed that the germicidal action of the blood of certain animals was due to its great alkalinity.

Metchnikoff<sup>9</sup> attributed the ability of the blood to destroy bacteria to the power of certain of the white cells of the blood, the polymorphonuclear leucocytes, to devour the micro-organism, to their *phagocytic* power, as he termed it. That these cells can and do act in this way at times can not be denied, and nothing is more attractive than to watch one of them surround and engulf a malarial parasite in the blood on the warm stage. That the true germicidal property of the blood does not lie in this action of the leucocyte was shown some time after by Buchner, who killed these cells by freezing and then thawing the blood, when its power to destroy micro-organisms was found to be undiminished. Buchner<sup>10</sup> further came to the conclusion, from a series of experiments that it is not necessary to review here, that the actual germicidal substance in blood was its serum-albuminous constituent.

Vaughan,<sup>11</sup> in a paper read before the medical section of the First Pan-American Medical Congress, in 1893, showed positively that this conclusion of Buchner must be erroneous, since it is known that whatever the substance in the blood is that possesses germicidal properties, it is not affected by peptic digestion, while serum-albumin is readily digested, as is well known.

From that time to the present numerous papers on this most important subject have appeared, and as many attempts have been made to clear up the question by the various theories that have been advanced.

It would only be necessary to mention the names of some of the investigators to show that the greatest



activity has prevailed in this particular field of research. Among those who have followed Buchner may be mentioned Pruden,<sup>12</sup> Rovighi,<sup>13</sup> Pekelharing,<sup>14</sup> Hankin,<sup>16</sup> Bitter,<sup>17</sup> Christmas<sup>18</sup> and a score of others, each with his own set of facts and consequent deductions. Suffice it to say that to each one there seemed to be some reasonable objection that rendered it probable that the true nature of the germicidal substance remained in doubt up to a very recent date.

Vaughan, in the paper just cited, makes a careful survey of the literature of the subject, analyzes the work of the most important investigators and comes to the following conclusions:

1. That serum-albumin as described by Buchner is not the germicidal substance in blood serum, since it is readily converted into peptone by peptic digestion, and still the germicidal property of the blood is unaltered. Peptone further is favorable to the growth of bacteria and is used in preparing nutrient media.

2. That the germicidal substance must belong to the proteids, otherwise it would be difficult to explain the fact that a temperature of 55 degrees C. renders blood serum inactive.

3. That the only proteid likely to be present in blood serum and which is not destroyed by peptic digestion is *nuclein*.

He then asks the following questions: 1. Is there a nuclein in the blood? 2. Has this nuclein, if present, any germicidal properties?

Without reviewing Vaughan's work, it is only necessary to say that he succeeded in isolating a substance from the blood which is undoubtedly a nuclein, and that he and a number of others have demonstrated the fact that it has very decided germicidal properties. It is therefore safe to say that we may now consider blood plasma to be germicidal by virtue of the *nuclein* that it contains.

Having then reached this important conclusion let us briefly inquire into the nature of this substance. What is nuclein?



The term nuclein was originally applied to a peculiar phosphorus-containing substance which was isolated from pus. Chittenden,<sup>19</sup> speaking of this says, "it was discovered that this or related substances were widely distributed through nature in both the animal and vegetable kingdoms wherever nucleated cells occurred."

From a physiologic standpoint nucleins may be considered to form the greater part of the chemie substance found in the nuclei of all cells, that substance which shows such a strong affinity for certain coloring agents, and which is sometimes spoken of as chromatin. It is, speaking broadly, "that constituent of the cell by virtue of which the histologic unit grows, develops and reproduces itself." It will be readily seen, therefore, that nuclein must be very widespread and that relatively there will be more of it found in those tissues or structures that are the richest in cellular elements.

Chemically, the nucleins are highly complex proteid bodies, of weak acidity, containing a certain amount of phosphorus. They are insoluble in weak acids and in alcohol, but are soluble in dilute alkalin solutions, the action of the alkali being to liberate free nucleinic acid, or to separate it from the albuminous matter with which it is in combination. Thus viewed a nuclein "is simply a combination of some form of proteid matter with a nucleinic acid" (Chittenden<sup>19</sup>). Further, the nucleinic acid can be prepared from any form of nuclear material, but it is found to be most easily gotten from yeast-cells. It is "characterized by its large percentage of phosphorus, as much as 9 per cent. having been found in some forms" (Chittenden). This acid can again be decomposed by the action of dilute mineral acids, and when so treated gives as cleavage products, various kinds of nucleinic bases, the difference depending on the nature of the tissue or substance from which the nuclein was originally derived. Thus, for example, the nucleinic acid from yeast-cells yields, when treated



with a dilute mineral acid, four distinct substances—bases, while that from the thymus gland of calves yields only one base—adenin. These facts are mentioned because, as is well-known, there is some close relation existing between this group of substances, the decomposition products of the nucleins, known as “xanthin bases,” and the formation and excretion of uric acid, a question that we will not attempt to discuss at the present time.

Since all nucleins contain phosphorus, and since it seems that the greater the amount of phosphorus the more active is the nuclein, the amount of phosphorus which any given sample of this substance contains may be taken as an index of the amount of nucleinic acid present in the preparation.

The various commercial articles now on the market have been found to vary considerably in the amount of nucleinic acid that they contain. According to a recent report of Professor Chittenden,<sup>19</sup> that manufactured by the firm of Parke, Davis & Co., under the name of “Nuclein Solution from Yeast” contains the most phosphorus, and therefore is the richest in nucleinic acid. It is the preparation used by us in the experiments that form the basis of this communication.

Having seen what nuclein is, let us inquire into the nature of its physiologic actions. The use of this substance as a therapeutic agent is of such recent date, and is based on such a narrow margin of known facts regarding its action, that there is comparatively little known as to its full action on the diseased economy.<sup>50</sup> Löwitt<sup>20</sup> some time ago made the statement that, by subcutaneous injection of nuclein in animals in the ratio of 1 per cent. per kilo. of weight, he quickly produced very dangerous symptoms, such as threatened cardiac and respiratory failure. It is impossible to say what preparation he used, or how it was prepared, but most certainly our observations and those of others have been just the opposite, and we have never seen, either in animals or man, any harmful symptoms



whatever even from considerable doses. That harmful results might occur if excessive doses were used may be conceived, but the same holds good for nearly every therapeutic agent, each having its proper dose and indications for its use, and there would be no occasion whatever to use doses of this preparation of such size as to produce even the mildest signs of danger. Certain effects have been noted in the treatment of tuberculosis and other diseases, with this remedy, but it is not the province of this paper to discuss these, which belong more properly to a consideration of the therapeutic uses and effects of nuclein. Whatever the other effects may be, however, there is one result of the administration of nuclein, either by the hypodermic method, or per os, that appears to be quite constant, and on which its therapeutic value is supposed to depend in the treatment of the various forms of bacterial disease. This effect is the *apparent production of a leucocytosis*.

It will be recalled that in the opening paragraph we stated that, could that substance be discovered by virtue of which the blood possessed germicidal properties, it might with reason be hoped that some means could be devised by which this valuable property could be artificially increased at will. Since the germicidal property of normal blood resides in a substance known as nuclein, and since this is derived from the nuclei of certain cells of the blood, the white corpuscles, it is believed that by increasing the number of these cells very materially the germicidal power of the blood plasma may be considerably heightened.

We have just said that one of the constant results of the injection of nuclein, or of its administration per os, was the production of an apparent leucocytosis, meaning by that term an actual increase in the total number of leucocytes per cubic millimeter, circulating in the blood. The word "apparent" is here used advisedly because it is claimed by some writers that the increase is only apparent, not real. It can readily be seen that on the determination of this im-



portant point the value of this or of any similar preparation will rest. If the results following the administration of nuclein consist only of a re-arrangement of the leucocytes in the different portions of the circulation and not of an actual increase in the number of the same, we can not hope by its use to augment the germicidal power of the blood, since we can not increase those bodies, the white corpuscles, that produce the germicidal substance.

According to those who hold the view of leucocytosis just mentioned, the effect of an injection of nuclein, or of any substance acting as it does, is to cause a determination of the leucocytes to the peripheral circulation and away from the circulation in the internal vessels. How this is supposed to be accomplished we will see later. On examining a drop of blood drawn from the finger tip, or from the lobe of the ear, as is the usual custom some hours after the administration of nuclein, it is found that there is an increase in the number of leucocytes, chiefly, though not entirely, confined to the polymorphonuclear variety, which fact is explained in the way just mentioned, it being denied that the total number of leucocytes is at all increased. According to this view then, what we have been taking for an increase in the number of cells is in reality nothing more than an affair of distribution, and we would have to alter our definition of leucocytosis accordingly.

Cabot,<sup>21</sup> in his recent book on the blood, defines leucocytosis as: "An increase in the number of leucocytes in the peripheral blood over the number normal in the individual case, this increase never involving a diminution in the polymorphonuclear varieties, but generally a marked absolute and relative gain over the number previously present." This definition he follows up by the statement that whether or not it be true that a leucocytosis is only an affair of distribution "it is accurate to say that in the drop which we draw (whether also in the internal organs or not) the leucocytes are present in increased num-



bers per cubic millimeter." This leaves us in doubt as to Cabot's own view, though strongly inclining us to believe that while he has not so expressed it in as many words, he holds the opinion that in leucocytosis there is an actual increase in the number of cellular elements throughout the circulation.

Ziegler,<sup>22</sup> also defines leucocytosis as an increase in the number of white corpuscles circulating in the blood and points out that these may come either from a new production of cells in the cell-forming organs, from a proliferation of the cells in the circulating blood, or further that the increase may under certain circumstances be due simply to the discharge into the circulation, from the lymph glands, of large numbers of ready formed, partly matured cells.

The arguments advanced in support of the view that leucocytosis is but an affair of distribution are worthy of very careful examination. There are two chief ones on which the theory rests. In the first place it is claimed that the apparent increase in the number of cells occurs chiefly in the polymorphonuclear variety which, if we accept Uskow's theory and classification, are the mature elements. If it is true that an actual new formation of cells takes place as the result of the injection of nuclein, the claim is made that we should find the greatest percentage increase in the mononuclear or young elements. In the second place it is claimed that an examination of the blood shows that while there is an apparent increase in the number of cells in the peripheral vessels the central circulation shows a corresponding paucity of cellular elements.

These two arguments, if based on careful experimental evidence, would be very strong indeed and would point to the accuracy of the conclusions drawn. It was partly with a view to clearing up this question that the experiments which form the basis of this paper were undertaken, and we believe that from the results we have obtained, and from those obtained by others, especially by Buchner and Hahn in a different



direction, which however bears strongly on the point in question, that we have reason to believe the leucocytosis following the administration of nuclein to be one in which there is an actual increase in the number of white corpuscles in the circulation. To what process this is due we are not able to state, though we are inclined to the view that there is proliferation within the cell-forming organs and probably in the circulation as well.

Before detailing our experiments let us examine more closely the two chief arguments advanced in support of the other view of leucocytosis, that first mentioned.

In examining a drop of blood, drawn, we will say from the finger of a person some hours, or a day or so, after an injection of nuclein, it is readily seen that the white corpuscles are present in increased numbers, and that the largest percentage increase is in the polymorphonuclear variety. This fact can not be disputed, and since, as Ehrlich<sup>23</sup> has pointed out, the blood-cell-forming organs discharge into the circulation only the small mononuclear or young elements, it seems at first difficult to explain the presence of so many of the older mature cells. The question however resolves itself really into one concerning the length of the life-cycle of a leucocyte. How long may it take for a young element to pass through the various stages in its development and become matured? Is it not probable that the mistake has been made in examining the blood too long after the injection of nuclein, so that time enough has elapsed to allow the young elements formed as a result of the injection to mature? If this is the case the first argument advanced loses its weight and becomes in reality an argument in favor of the other view.

In the course of all of our experiments we found that within so short a time as from five to ten minutes after the injection of nuclein there was a noticeable increase in the number of leucocytes in the peripheral as well as in the central circulation, and,



moreover, that the greatest percentage increase was not in the mature, but in the young varieties. If it is possible then for an actual reproduction to begin within so short a time as five to ten minutes after an injection, is it not probable that the majority of these elements can pass on to the mature stage after the lapse of a day, or even of some hours? We think it is and that herein lies the explanation of the fact that when a drop of blood from the peripheral circulation is examined some hours after the injection, the increase is seen to be chiefly in the polymorphonuclear forms. The fact, also, that in our experiments we found that the small young elements were the first to be increased in the largest proportion is consistent with the view that the leucocytosis produced by nuclein at least, consists of an actual increase in the total number of white cells, and bears out the statement of Löwitt<sup>20</sup> that following an initial stage in which there seems to be a lowering of the number of corpuscles (which phenomenon we have not attempted to study), there is a development of young mononuclear forms which gradually pass through the developmental stages into mature elements.

Goldschneider and Jacob<sup>24</sup> also, though they disagree with Löwitt in some of his conclusions, support him in the statement that the injection of certain substances produces an increase in the number of leucocytes most noticeable in the mononuclear forms.

Romer<sup>25</sup> likewise takes this view of the process and goes so far as to say that he believes the reproduction to take place within the circulation as well as in the blood-cell-forming glands.

In attempting to explain how it is that the injection of such a substance as nuclein causes a determination of leucocytes to the peripheral circulation and away from the that in the internal vessels, it seems to us that the advocates of this theory disclose its weakness as they do in no other way.

While many writers attempt no explanation others, as Wells,<sup>26</sup> in a recent article states that according to



his belief "the key to the whole problem is to be found in the principle known as chemotropismus, or more commonly as chemotaxis." According to this author the effect of an injection of nuclein is to exert a chemotactic action, the result of which is the attraction of the polymorphonuclear leucocytes from the central to the peripheral circulation.

We fail to see how such an action could take place, even granting that nuclein possessed chemotactic properties, for is it to be supposed that after being injected subcutaneously the substance will remain in the peripheral circulation? Will it not naturally circulate freely throughout the system? This at least is what one would expect, and doing so it would then exert its chemotactic influence, if it had any, in all portions of the circulation, and the results would be *nil* because the various attractive forces would neutralize one another. In fact, this chemotactic explanation does not seem to us to be any explanation at all.

Aside from all the foregoing facts that seem to us to strongly militate against the idea that leucocytosis is but an affair of distribution there is another fact, well authenticated, that also points conclusively to the incorrectness of this view.

Referring again to a paragraph in the early part of this paper, it was stated that the germicidal property of the blood resided in its nuclein and that the richer in cells a part was, the more its content of nuclein, and by the same reasoning the greater its germicidal power.

Buchner, working on this question, found that fluids rich in cells possessed a greater germicidal power than did the normal blood of the same animal. More recently Hahn,<sup>27</sup> working under Buchner and following the suggestion contained in the above observation, has attempted to increase the germicidal power of the blood plasma by producing an artificial leucocytosis. Hahn found that the blood plasma of animals treated by hypodermic injections of nuclein solution possessed germicidal power considerably greater than the blood of the same animals had pos-



sessed before the injections. Had the effect of the injections been to cause a determination of the leucocytes to the peripheral circulation only, and not an actual increase in the total number of the same it is very difficult to explain Hahn's results which, therefore, seem to us to point very conclusively to the fact that the leucocytosis produced by this substance at least consists of an actual production of new leucocytes, increasing the number of nuclein-producing bodies and in consequence heightening the bactericidal power of the blood.

Among those who are cited by Wells as supporting the view that we believe to be incorrect may be mentioned X. Reeder,<sup>28</sup> who says, "as to the origin of leucocytosis we have on the ground of experimental examinations *no good ground* to believe that it is due to an increased supply of white blood corpuscles from the blood-making organs, still less to an increase of the same in the blood, or to an abnormal collection of wandering cells. It must be considered as more probable that the leucocytosis is based only upon an *insignificant increase* of the entire number of white cells circulating in the blood, in that there occurs an abnormal distribution of the same in the different vessels in favor of the periphery."

A careful examination of this statement of Reeder shows that, while in the first paragraph he states positively that there is *no evidence* of the new formation of white cells *whatever*, he goes on to say in the next that there is an insignificant increase in the total number. He would seem then to admit that a production of new cells could and did take place, even if in small number only, and in so far to contradict himself. If it is admitted that it is possible for a reproduction to take place, why not in considerable numbers as well as in small? The important fact is in admitting that they *can and do reproduce*.

Schulz<sup>29</sup> is also mentioned as an advocate of this view. In one experiment made by this observer, and cited by Wells, he injected into the ear of a rabbit



2 c.c. of bacterial protein and immediately withdrew blood from the other ear, in which he found on counting the leucocytes only 3,300 as against 11,100 before the administration of the protein. As rapidly as possible, counts were made from several internal vessels, the average of which was 21,500. From these figures he draws the conclusion that the decrease and therefore also the subsequent increase, is only apparent, and that at first the leucocytes left the peripheral circulation for the central, and afterward the central for the peripheral. He evidently overlooked the fact, that had he estimated the total number of leucocytes before and after the injection he would have found that in the very short time that it took him to make the experiment there had been an actual increase in the whole number of 1,300 white cells per cubic millimeter, even though the cells at the time of the count were unevenly distributed.

From all the facts that we have cited it would appear that we are justified in looking on the change in the circulation that follows an injection of nuclein as a leucocytosis in which there is an increase in the total number of white corpuscles.

The experiments that we made were all upon medium-sized dogs, and while we will not detail each one we will give the steps pursued and a few of the results which are typical of all, since with very slight variations practically the same results were obtained in all cases. Before putting the dog under ether a count was made of the leucocytes in the peripheral circulation. Ether was then administered and the same was done for the peripheral and central circulations. We made the count of the peripheral circulation before the administration of ether, because we found that the ether alone produced some increase in the number of leucocytes, the amount seeming to depend on the rapidity or slowness with which the animal succumbed to the anesthetic; a greater change was noted in those dogs that struggled the most and required the most ether. After making the counts



already mentioned a hypodermic injection of nuclein solution (1 per cent. P. D. & Co.), varying from 25 to 45 minims in the different experiments, was given and counts made from both the peripheral and central circulations every five minutes thereafter up to thirty-five minutes in some cases. Care was taken to allow the animal to bleed but little, and as slight disturbance was produced by the operation as possible.

The leucocytes in the blood withdrawn were stained with a weak solution of methyl violet in normal salt solution and the results of the various counts tabulated. In every instance ten counts at least were made and the average taken. The entire field of the blood counter was counted every time. In this way it was hoped to reduce the limit of error as much as possible. The general results of these experiments may be summed up as follows:

1. The administration of ether alone causes an increase in the number of leucocytes over that found in the circulation before its administration. The amount of the increase seems to depend on the amount of ether used and the time required to anesthetize the animal.

2. Before the administration of nuclein the counts showed that the number of leucocytes in the peripheral and in the central circulation was practically the same, varying only within the limit of error, or slightly over, sometimes one way sometimes the other.

3. Following the administration of nuclein solution there was immediately, that is by the end of five or ten minutes, a noticeable increase in the number of leucocytes *in both the central and peripheral circulation.*

4. At this time the percentage increase was most marked in the young mononuclear forms, which in some instances rose as high as 60 per cent. of the whole within fifteen minutes, while at the same time the proportion of polymorphonuclear elements was proportionately low.



5. The longer after the injection of nuclein the greater was the actual increase and the number seemed to steadily rise *in both the peripheral and central circulation.*

As the title of this paper indicates, it is but a preliminary study and we recognize the fact that there are a large number of questions, upon which we have not attempted to touch, that are of the greatest interest and importance. One of these questions relates to the condition of the blood a day or two after the injection of nuclein. We believe, from certain facts that we have already stated, and from other observations that we have made, that we will find a gradual increase in the proportion of polymorphonuclear elements beginning an hour or so after the injection and gradually increasing, and that as this rises there will be a falling off in the proportion of mononuclear forms as they gradually mature. Work on this point will be begun in the near future, as well as on some others that have suggested themselves to us.

The following tables taken from our record book give the figures in some of our experiments and the percentages calculated from them. It is from these figures that the conclusions just stated were drawn. A comparison of the following tables shows that the increase in the number of leucocytes begins within a very short time after the injection and steadily rises, and that the greatest percentage increase is in the small mononuclear elements.

Table 1.—Count of the leucocytes in the *peripheral* circulation of a dog before the administration of nuclein and without ether :

Varieties.	Per- centages.	Average total No. of leuco- cytes per c.mm.
Small mononuclears . . .	10.87	9,200
Large mononuclears . . .	26.07	
Polymorphonuclears . . .	63.06	

Table 2.—Count of the leucocytes in the *peripheral* circulation of a dog under ether and five minutes after an injection of 45 minims of nuclein solution :



Varieties.	Per- centages.	Average total No. of leuco- cytes per c.mm.
Small mononuclears . . .	42.30	15,600
Large mononuclears . . .	12.82	
Polymorphonuclears . . .	44.87	

Table 3.—Count of the leucocytes in the *peripheral* circulation of a dog under ether and twenty-nine minutes after an injection of 45 minims of nuclein solution :

Varieties.	Per- centages.	Average total No. of leuco- cytes per c.mm.
Small mononuclears . . .	39.23	20,400
Large mononuclears . . .	18.62	
Polymorphonuclears . . .	40.18	
Eosinophiles . . . . .	1.96	

A comparison of the following tables with the foregoing will show that the increase in the number of leucocytes occurred in the central circulation as well as in the peripheral and was not therefore an "affair of distribution."

Table 4.—Count of the leucocytes in the *central* circulation of a dog under ether and before the administration of nuclein solution :

Varieties.	Per- centages.	Average total No. of leuco- cytes per c.mm.
Small mononuclears . . .	15.58	15,400
Large mononuclears . . .	18.18	
Polymorphonuclears . . .	66.23	

Table 5.—Count of the leucocytes in the *central* circulation of a dog under ether and five minutes after an injection of 45 minims of nuclein solution :

Varieties.	Per- centages.	Average total No. of leuco- cytes per c.mm.
Small mononuclears . . .	34.79	18,400
Large mononuclears . . .	19.56	
Polymorphonuclears . . .	44.56	
Eosinophiles . . . . .	1.08	

Table 6.—Count of the leucocytes in the *central* circulation of a dog under ether and thirty-five minutes after an injection of 45 minims of nuclein solution :

Varieties.	Per- centages.	Average total No. of leuco- cytes per c.mm.
Small mononuclears . . .	50.90	22,000
Large mononuclears . . .	14.54	
Polymorphonuclears . . .	33.64	
Eosinophiles . . . . .	.91	



By comparing Table 1 with Table 4 and Table 7, which follows, it will be seen that the effect of ether alone is to cause an increase in the total number of leucocytes. There was no count made of the central circulation before the administration of ether, as we did not care to subject the animal to the pain of the operation. The evidence is very clear, however, that there is the same increase in the number of corpuscles in that portion of the vascular system following the anesthetic as there is in the peripheral portion.

Table 7.—Count of the leucocytes in the *peripheral* circulation of a dog after the administration of ether and before the injection of nuclein solution :

Varieties.	Per- centages.	Average total No. of leuco- cytes per c.mm.
Small mononuclears . . .	8.82	13,600
Large mononuclears . . .	20.50	
Polymorphonuclears . . .	67.64	
Eosinophiles . . . . .	2.94	

The figures given in all the preceding tables are taken from the same experiment. This was only one of a large number and each showed practically the same changes, the figures varying, the increase in the number of leucocytes being greater in some and less in others. The number of white corpuscles varies normally in different dogs and each one has its individual ability to react to the stimulation of the nuclein injected, this reaction however being present in every animal experimented with.

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- 30 This paper was written before Dr. Vaughan's recent article on the use of nuclein in cases of pulmonary tuberculosis appeared. The authors have themselves used it in a large number of such cases with apparent marked beneficial results. These it is expected will be reported later.



