STUDIES ON THE BACTERICIDAL AND AGGLUTINATIVE POWER OF SERUM AND PLASMA OF NORMAL AND PULLORUM INFECTED TURKEYS

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Introduction

There have been numerous reports in the literature regarding the bactericidal property and the inhibitory zone of serum. Much work has been done by others on normal animal serum using various species of organisms. The bactericidal action of each serum varies with each species and strain of organisms. Since the bactericidal action varies toward each species of organisms, it is necessary to test the specific serum and plasma on specific organisms.

The object of this experiment was to investigate the bactericidal action of turkey serum and plasma on Salmonella pullorum. First, observations were made on the killing power of normal turkey serum and plasma and then on the killing power of infected turkey serum and plasma. The agglutinating titers, and blood cell counts were also observed to see what influence they have on bactericidal action. The results explain why the plasma of infected birds had less bactericidal action than normal plasma, and why prozones occurred in the lower agglutinating dilutions.

Review of Literature

The observations of Nuttall (1888) and many others first contributed to our knowledge of serum reactions. It has been recognized that the bactericidal property of serum is a variable one, differing according to the animal species and the type of micro-organism concerned. There has been some uncertainty regarding the specificity or non-specificity of natural bactericidal effects. Muir and Browning (1908) reviewed the literature on this subject and studied the specificity of these reactions by absorption methods. They found that treatment of a normal serum with increasing amounts of bacterial suspension produced first a diminution of the bactericidal action towards the homologous bacterium, and also a decrease in the effect of natural complement-fixing and agglutinating antibodies. This suggested the likelihood that the bactericidal effects of normal serum may be due to multiple specific antibodies sensitizing bacteria to the lytic action of complement.

Thjötta (1919) showed that during immunization there is produced along with the antibodies, a complement-inhibiting substance which he believes to be separate and distinct from agglutinins, precipitins and bactericidal amboceptor. If sufficient dilution of the serum is made

and if extra complement is added, the immune serum will show bactericidal action, whereas undiluted, fresh immune serum, mixed with the homologous organisms, exhibits little if any bactericidal effect.

It was shown by Georgevitch (1926) that killed organisms incubated with serum neutralized non-specifically the bactericidal action of the serum. It is noteworthy that the neutralizing substance acts at 0°C. and is more active at 37°C. This agent, irrespective of the organism from which it is produced or the serum used for the bactericidal test, affects strongly the bactericidal antibodies for certain organisms, and is less active towards others.

The complements of different animal species were interchangeable in these bactericidal reactions and certain observations recorded led him to assume that differences in the bactericidal properties of sera towards particular organisms depend on variation in the antibody rather than in the complement.

Gordon and Wormall (1928) have shown how bacteriolysis of Shigella dysenteriae by normal guinea pig serum depends on the combined action of complement and a thermostable factor removed from the serum by absorption with organisms. The question is further complicated by the fact that different mechanisms may be concerned in the bactericidal

action of normal sera and that the factors involved may vary with different organisms.

Pettersson (1928) classified the bactericidal agents of serum into alpha lysins and beta lysins. The former apparently represent the complement acting with a sensitizing agent analogous to an immune body. The latter, according to Pettersson consist of a stable "activating" agent (resisting a temperature of 63°C. for a half hour) and an activable principle which unites with the bacteria in the presence of the activating agent.

Knarr (1929) showed how leucocyte extracts kill such organisms as streptococci, staphylococci, pneumococci, and Bacillus anthracis, whereas the alexin of serum acts on Salmonella typhosa, Escherichia coli, and Vibrio cholerae.

Finkelstein (1931) has done much work on the bactericidal action of serum, and he summarized his results as follows:

1. An analytical study has been made of the mechanism of natural bactericidal action by the serum of various animals towards certain organisms exhibiting the maximum reactivity to this effect.

2. The serum-complement has no bactericidal action by itself. An antibody-like agent invariably acts as an intermediary agent, "sensitizing" the particular organism to the action of the complement and is capable of being "absorbed" by it from serum at 0°C.

3. This sensitizing agent is stable at 55° C. but labile at 60°-65°C. In this respect it resembles natural hemolysins and agglutinins, but contrasts with the more stable immune antibodies and the more labile nature of the complement-fixing antibodies.

4. The absorption tests demonstrate the high degree of specificity of these natural bactericidal antibodies for particular bacteria.

5. A non-specific extracellular substance occurs in bacterial cultures which may neutralize or inhibit these antibodies, and interferes with their sensitizing action even at 0°C. This substance is liberated in large amounts in cultures heated to 120°C.

In 1932, Finkelstein demonstrated that the bactericidal property of normal serum towards gram-negative bacteria is labile at 55°C. for 30 minutes; the factors responsible for the corresponding effect on gram-positive bacteria are stabile at this temperature. Thus the gram-negative and gram-positive organisms are acted on by separate agents, the "thermo-labile" and "thermostabile" bactericidins respectively. Bactericidal effects are more frequent and pronounced towards the gram-negative than toward the gram-positive bacteria. The "thermolabile" bactericidin consists of complement and a sensitizing antibody. The lability of the bactericidin is due to the

lability of the complement. The antibody is stable at 60°C. and specific for the particular organism acted on. The "thermo-stabile" bactericidin in undiluted serum withstands a temperature of 57.7°C. though labile at 60°C; its lability is considerably increased in diluted serum and in slightly alkalinized serum though unaltered by slight acidity.

The work of Gordon (1933) demonstrated that the absorption of both normal unheated and heated sera by dead bacteria fails to yield any evidence of the existence of a series of specific antibodies in serum. The loss of bactericidal power consequent upon absorption is never specific for the absorbing organism but is always general.

Mudd (1933) showed that sensitization by serum renders various dissimilar bacteria similar with respect to their surface properties. This convergence of surface properties is carried further by homologous immune than by heterologous or normal sera, and the homologous immune sera are effective in higher dilutions.

The work of Gordon and Johnstone (1940) also showed that the absorption of a normal serum by a series of strains of one organism causes a general diminution in bactericidal power for all the strains, but there is a more striking diminution for the strain with which the serum was absorbed. Three strains of Micrococcus catarrhalis were used to absorb a guinea pig serum. They

demonstrated that the complement titer of guinea pig serum was high, that of human serum low and that of rabbit serum still lower. The results show low bactericidal action of human serum on the gonococcus, whereas guinea pig serum with a higher and rabbit serum with a lower complement titer were both markedly bactericidal. In this experiment one human serum had no bactericidal action on Vibrio cholerae but another human serum killed Vibrio cholerae in one hour. The rabbit serum, which had a lower complement titer than the guinea pig serum, was again the more bactericidal, and inactivation of complement completely destroyed the bactericidal action of both sera. They showed that in many species specific antibodies can be individually absorbed or that there is a general bactericidal antibody which can be so modified by contact with a large excess of any particular organism or strain as to render it specifically inactive for that organism or strain.

Agglutination and Inhibiting Zone

The older hypothesis, suggested by Eisenberg and Volk (1902) accepts Ehrlich's conception of the agglutinin as being made up of an antibody-bacteria binding portion (haptophore) and a flocculating portion (zymophore), and assumes that by heating or aging, some of the agglutinin is so modified (agglutinoid) that the

clumping component is destroyed without, however, affecting the binding portions. As a result, the agglutinoid may still unite with the bacteria but does not produce flocculation. In order to explain the inhibition effect in high serum concentrations, it is assumed that the agglutinoid in these concentrations has a greater affinity for bacteria and is therefore bound to them to the exclusion of effective agglutinins.

The second hypothesis is put forward by Zinsser (1923) as follows:

Agglutinoid zones are analogous to zone phenomena of other antibody reactions, notably the precipitin reaction, and are definitely dependent upon quantitative union between antigen and antibody and have nothing to do with deterioration of antibody by heat or otherwise.

In various colloid precipitations in which serum is involved, moderate heating of the serum will strongly reduce its ability to precipitate a suspension. When normal serum is heated it is likely that there is a change in its colloidal state producing a certain amount of colloidal protective property in the serum. In reactions between bacteria and anti-serum, it is likely that the antibody carries into union a not inconsiderable amount of active serum constituents. The so-called specific action of the agglutinoid is probably due to the fact that the antibody carries into union with the

bacteria inactive protein which is colloidally protective by virtue of the heating.

Shibley (1924) summarized his results as follows:

1. Immune agglutinating serum possesses a specific charge-reducing effect which is quantitatively related to the agglutination titer of the serum. This effect is lost when the serum loses its agglutinating power; that is, after adsorption of agglutinin by homologous bacteria. Adsorption by heterologous organisms does not affect this property.

2. A highly protective non-agglutinating serum did not show this specific charge reducing effect.

A few years later he worked on the mechanism of the agglutination of bacteria. He concluded that the process of sensitization by agglutinating serum consists of a selective coating of the bacteria by the globulin of the antibody. This film formation causes the bacteria to take on the characteristics of particles of denatured globulin. Subsequent agglutination of the coated bacteria follows the laws governing the flocculation of particles of denatured protein by electrolytes.

Shibley (1929) heated serum from 55°C. to 76°C. for 10 minutes, then made agglutination tests. He found that the inhibition zone (prozone) begins at 64°C. The zone then widens to reach a peak at 66°C. to 69°C. Above this the zone narrows and disappears at 72°C. Coincident with

this narrowing and its loss, there is a corresponding drop in the agglutinative titer. At 76°C. all agglutination disappears.

He proposed the hypothesis that the inhibition zone is caused by a modification of the agglutinin; (a) it still retains its binding power although when union has taken place the agglutinin-bacteria complex fails to clump, and (b) it has a greater affinity for the bacteria than the unchanged agglutinin.

Now, when the heating level is further raised, it will be seen that the inhibition zone is reduced and then disappears. He explains this on the assumption that this higher heating further modifies the modified agglutinin so that it now loses its binding power.

When serum was heated to 70°C. for 10 minutes and titrated at pH 7, prozones were wider than those of lower pH. At pH 5.4 no prozones were present (serum heated at 74°C.)

With 16 billions of organisms per cc in the titrating mixture, no prozone appeared. Titrating serum antigen mixture containing 8 billions of organisms gave some prozone while tubes containing 2 billions of organism gave wider prozone. He also absorbed serum with 64 billions organisms per cc and produced no prozone but absorbing with 2 billions of organisms per cc gave a prozone.

Eagle (1930) suggested that agglutinating and precipitating antibodies are a specifically altered fraction of the serum globulin. The antigen-antibody complex, whether it be sensitized red cells, agglutinated bacteria or precipitate, formed by a soluble protein and the corresponding antiserum, contains this antibody globulin, demonstrable chemically, immunologically and by a change in the cataphoretic, flocculating, interfacial and complement-fixing properties of the antigen towards those of the protein with which it has combined.

In the case of the cellular antigen, this antibody is present as an invisible film of specifically adsorbed protein, while in the precipitation reaction, it may constitute the bulk of the material formed. In both cases the originally hydrophilic globulin has become water-insoluble (denatured) upon combination with antigen. This change in properties is not a phenomenon peculiar to the immune reactions but is a commonly observed and as yet unexplained property of adsorbed proteins, responsible for their sensitizing effect upon other-wise stable colloidal suspensions. It is suggested that in the case of the immune reactions, this denaturation of the antibody globulin is due to the fact that its specificity is determined by hydrophilic groups. When these combine with antigen, hydrophobic groups necessarily face the water phase, determining the surface properties of the antigen-antibody complex. But when normal serum

protein is adsorbed, since there are no groups with a specific affinity for the antigen, the molecules naturally orient themselves at the interface so that the hydrophilic groups face the water, and the adsorbed protein acts as a protective film.

There are therefore three factors which determine specific flocculation: (1) the hydrophilic antigen is covered, with (2) a film of immune globulin, denatured by its combination with antigen. In the absence of electrolytes the charge due to the ionization of this protein suffices to prevent aggregation. Minute concentrations of (3) electrolytes, however, depress this surface charge below the critical value necessary for stability. The resultant aggregation is therefore primarily of the immune globulin surfaces, and only incidentally of the associated antigen.

With insufficient immune-serum, only a very small portion of the cell surface is covered with antibody globulin; most of the impacts are between hydrophilic antigen surfaces, ineffective in producing cohesion. The more immune serum, the greater is the proportion of antigen surface covered with the sensitizing denatured protein, and the correspondingly greater the proportion of effective impacts.

The optimum hydrogen ion concentration for flocculation is intermediate between that of the original

cell and that of the antibody globulin, shifting towards the latter as the degree of sensitization is increased (more extensive antibody film). At the optimum reaction, ionization takes place and therefore the surface charge is minimal. No added electrolytes are necessary to produce aggregation. In a more acid or a more basic reaction, the surface charge, due to the ionization of the adsorbed protein, causes a mutual repulsion of the particles; but traces of electrolytes depress this charge and allow the cohesion of the denatured antibody films. The flocculating ion is always the one opposite in charge to the ionized protein, and its flocculating efficiency increases enormously with increasing valence. The further from the isoelectric zone, the greater is the degree of ionization and the more electrolytes are necessary to depress the surface charge below the critical value.

Jones and Orcutt (1934) reported that when two agglutination inhibitory sera specific for Brucella abortus were added to a strong B. abortus agglutinin, agglutination was inhibited and a prozone developed. Bacteria not agglutinated in the prozone serum can be centrifuged and resuspended in the same mixture and remain in suspension. When the original supernatant is replaced with salt solution, agglutination usually occurs promptly although, where the concentration of prozone serum is considerable, an additional washing with saline solution may be required

to induce clumping. They think the failure to agglutinate may be attributed to the deposition on the surface of the deposited globulin film of a substance which reduces the cohesive properties of specifically sensitized organism.

Duncan (1937) states that the salt optimum determines the maximum combination of antibody with antigen and thus influences the quantity of agglutination measured by the highest effective serum titer. Antibody-antigen combination reaches its maximum at the salt optimum and it diminishes progressively as the salt concentration deviates in either direction from this optimum.

Wiener and Herman (1939) believe that the precipitative and agglutinative reactions take place in two separate stages: (a) a specific combination between the antigen and its antibody and (b) a non-specific stage of aggregation of the sensitized particles, in which electrolytes play a role.

The work of Pauling, Campbell and Pressman (1934) indicates that the forces responsible for combination and attraction of antigen and antibody molecules may be classified as electronic van der Waal's attraction, Coulomb attraction, attraction of electric dipoles or multiples, formation of hydrogen bonds, etc. The specificity of interaction of antigen antibody molecules

arises from their structural complementariness, which permits close contact of the molecules over sufficient area for these weak forces to cooperate in forming a strong antigen-antibody bond.

The weight of evidence indicates that further combination of the initial antigen-antibody complexes to form a precipitate is a specific rather than a non-specific reaction and is due to a continuation of the primary combination step to form a framework structure of alternate antigen and antibody molecules. Furthermore, it appears that both precipitating antigen and precipitating antibody must be multivalent or at least bivalent.

Wiener (1944) showed that the prozone phenomenon may be due to the presence in sera of a mixture of blocking and agglutinating antibodies.

Jenkins (1946) found that sensitized bacilli can release antibody at high temperature and take up more at low temperature. The released antibody is a globulin and is accompanied by another globulin which is not specific. The released antibody presents some characteristics which differentiate it from serum antibody.

Hayes (1947) favors the idea of the altered (by heating) physical properties of the cell. This alteration of the bacterial cell causes the colloid adsorbed from the serum to protect the organism from antibody action. He classified the prozones into 3

groups as follows: Prozone "A", occurs with unheated serum. Prozone "B", occurs with sera which have been heated. Prozone "C", occurs when heated S. typhi O suspension is mixed with unheated serum. On the basis of the results of his experiment, he proceeded to explain such interference as follows:

1. A primary non-specific adsorption of "albumin fraction" by the cell will prevent the fixation of agglutinin on the cell surface.

2. A secondary adsorption of "albumin fraction" by the agglutinin-cell complexes which inhibits aggregation.

Blood Cells and Their Response to Infection

Chandhuri (1927) found that the number of erythrocytes in a unit volume of blood is significantly higher in the sexually normal adult male than in the normal adult female of the fowl.

Hofmeister (1934) reported that leghorns with acute infection showed a sharp rise in the number of pseudo-eosinophile leucocytes, reaching a peak in 1 to 3 days. At the same time small lymphocytes decreased in numbers and returned to normal within 8 days. A moderate increase in large lymphocytes occurred 2 to 4 days after the increase in pseudo-eosinophils and returned to normal after 7 days. The percentage of neutrophils in inoculated resistant birds was somewhat higher than in non-inoculated resistant ones.

Biely, Jacob and Palmer (1935) found that the range of red blood cell count of birds was 1,805,000 to 3,845,000 per cu.mm. The mean red cell count of the male was significantly higher than that of the female but there was no significant difference between the mean leucocyte counts of males and females.

At the World's Poultry Congress in 1939, Roberts, Severens and Card reported that the number of lymphocytes is greater in resistant than in susceptible fowls.

The work of Scholes (1942) indicates that resistance more probably depends upon temperature differences than upon differences in the number of lymphocytes in the blood.

Materials and Methods

Blood cell counts. The method of Wiseman (from Olson, 1935) was used for the routine study of turkey blood. The diluting fluid consisted of 50 mg. of phloxine, 5 ml. of neutral formalin, and 95 ml. of Ringer's solution. The ordinary red blood cell diluting pipette was used. The blood was diluted 200 times. The filled pipettes were allowed to stand for several hours to allow the cells to take up the dye before the count was made in the hemocytometer. The ends of the pipettes were closed by stretching a heavy rubber band lengthwise around the pipette during the interval to avoid loss of fluid. The cells were counted in the usual manner; that is, the erythrocytes in 80 of the smallest squares were counted, and the result multiplied by 10,000 which represents the number of erythrocytes per cubic millimeter of blood.

The number of leucocytes was obtained by counting the acidophilic granulocytes which were specifically stained by phloxine in the entire ruled area of the hemocytometer (9 mm^2).

The differential count of leucocytes was made from the stained blood smears. Thin blood smears were stained with Wright's stain. A daily cell count was made on normal and infected turkeys.

Preparation of culture. A smooth strain of S. pullorum (isolated from a turkey) was used in this experiment. The organism was grown on nutrient agar slants for 24 hours at 37°C. The growth was removed by means of a sterile wire loop and suspended in sterile diluting fluid, which consisted of 0.05% tryptose peptone and 0.5% sodium chloride in distilled water. Ten ml. of this diluting fluid was usually used for each agar slant. The bacterial suspension was transferred into a sterile test tube, then thoroughly mixed and diluted to turbidity No. 2 of McFarland's nephelometer (For standard turbidity, refer to "Laboratory Diagnostic Methods" by Kolmer). Serial dilutions ranging from $8x10^{1}$ to $8x10^{9}$ were made from this suspension in the same diluting fluid. The number of organisms present was determined by plating 0.25 ml. or 0.1 ml. of 10^{-9} and 10^{-8} dilutions. The pour plate method was used. The nutrient agar was melted, cooled to 45°C. and poured into the Petri dish which was rotated to mix the content well before the agar solidified. When the agar was solidified the plates were incubated for 3 to 4 days at 37° C. and then the colonies were counted. The initial number of bacteria added to the blood plasma or serum from dilutions can be calculated by multiplying the number counted by the dilution factor.

Preparation of blood. Each bird was tested for S. pullorum infection by using the stained antigen rapid whole-blood test.

Blood for the bactericidal test was drawn aseptically from the wing vein of the bird and placed in a sterile bottle. For the tests requiring plasma, 0.1 ml. of sterile saturated sodium citrate solution for each 10 ml. of blood was placed in the bottles.

The plasma was separated from the whole blood by centrifuging at 1,800 r.p.m. for 25 minutes. The supernatant was poured off aseptically into another sterile tube. Serum was obtained by allowing the blood to clot after which it was centrifuged if necessary.

All bactericidal tests were made within 24 hours after collection of blood unless otherwise stated. During this period the blood specimens were kept in an ice box (4°C.).

Turkeys were immunized by injecting 1 ml., 2 ml., 3 ml., or 4 ml. of dead S. pullorum (8×10^{10} per ml.) suspended in 0.85% saline intravenously. Later, live S. pullorum was injected into the same turkeys intravenously. Repeated injections were given until the turkeys showed a high agglutination titer.

The bactericidal test. In order to show the maximum bactericidal activity of serum or plasma on S. pullorum, two methods of setting up the tests were used:

1. One ml. or 0.5 ml. of undiluted serum or plasma was added to a series of tubes to which was added the same volume of diluting fluid containing live organisms in varying numbers. 2. Serial dilutions of serum or plasma, in 1.0 ml. or 0.5 ml. amounts, were placed in the sterile tubes to which were added the same volume of diluting fluid containing a constant number of live S. pullorum. The tubes were shaken and incubated at 37°C.; the length of time varied with the experiment. At the end of the period of incubation, 0.25 ml. or 0.1 ml. of the mixture was taken from each tube and placed in a sterile Petri dish. Melted nutrient agar was cooled to 45°C. and 10 ml. amounts were then poured into each Petri dish. The contents were mixed by rotation and then allowed to harden after which the plates were incubated at 37°C. for 3 days. Colony counts were made and compared with those of the control tubes. The same procedure was repeated at the end of 4 hours, 8 hours, 24 hours, and 48 hours.

Table No. 1 shows how the dilutions were prepared and the amounts of S. pullorum suspension used.

The S. pullorum antigen was made by growing the organisms in large bottles. After several days of growth in nutrient agar at 37°C. the organism was removed with 1% phenol water. The bacteria were washed by centrifugation. The supernatant was poured off and

the organisms resuspended in diluent containing 0.25% phenol. The bacterial suspension was diluted to turbidity No. 1 of McFarland's nephelometer. This bacterial antigen was used for agglutination tests. The pH of this antigen was adjusted with a Beckman pH meter.

A serial dilution for the agglutination test was set up as follows: 1.9 ml. of the S. pullorum antigen was placed in tube No. 1 and 1.0 ml. in all the following 10 tubes, then 0.1 ml. of plasma was placed in the first tube. The content was mixed well and 1.0 ml. was transferred into the second tube, etc. See Table No. 2 for agglutination dilutions.

Results and Discussion

All conditions must be taken into consideration in order to obtain a true blood picture. Factors such as species, age, sex, temperature, food, and various abnormal conditions have direct effect on blood composition and cell count. So, in order to obtain accurate results, all turkeys should be of the same age, sex, etc. After taking all these important factors into consideration, a daily cell count was made on all turkeys to be used in these experiments. It was found that the average cell counts of 18 three-month old normal turkeys was as follows:

Total leukocyte count - 12,000 per cubic mm.
Total erythrocyte count -2,600,000 per cubic mm.
Total heterophil count - 43% of the total leucocyte
Total lymphocyte count - 51% of the total leucocyte
Total eosinophil count - 1% of the total leucocyte
Total basophil count - 2% of the total leucocyte
Total monocyte count - 3% of the total leucocyte

This agrees quite closely with the differential leukocyte count of Johnson and Lange (1939).

After a sufficient number of blood cell counts were made on normal turkeys, one half of these birds were infected with S. pullorum and daily cell counts were continued. It was found that 24 hours after in-

jection of S. pullorum there was a sudden increase of heterophils which remained at a high level for about three days, then began to drop quite rapidly. The percent increase of heterophils apparently depends on the amount of bacterial suspension used and the susceptibility of the turkey. The heterophil count usually returned to normal about six days after the administration of S. pullorum. The lymphocytes in this experiment, however, showed only a slight although rather irregular increase in most cases. The differential counts showed more young, large lymphocytes after the rise of the agglutinating titer. This may indicate a relationship to antibody formation. The eosinophils, basophils and monocytes were found to be increased somewhat, but not to any significant degree in this case. The erythrocyte count decreased somewhat after the administering of S. pullorum.

The total leucocyte count usually increased gradually after each successive oral administration of organisms as shown in Figures 2, 3 and 4, whereas there was a very sudden increase in total leucocyte count after the first intravenous injection of organisms. There was less variability following the later injections as shown in Figures 6 and 7. It stands to reason that infections occur more readily as a result of intravenous injection. The birds which were infected

orally generally showed low agglutinating titers whereas those which were infected intravenously showed very high agglutinating titers when the same dosage of organisms was used.

Bactericidal tests. The results obtained in this experiment indicate that the bactericidal action of plasma is somewhat greater than that of the serum. Just why that is the case is not yet known. However, plasma may have more antibody present than the serum. It is possible that during the process of coagulation some of the antibody may have been removed with the fibrin, thus causing a decrease in bactericidal action. Plasma is more like normal blood than serum and reacts more effectively. Therefore, all subsequent tests were made with plasma.

Tables 3 and 4 show that the serum and plasma of different normal turkeys vary with respect to bactericidal power. Tables 5 and 6 show that the plasma is more bactericidal than the serum. Plasma from the infected turkeys also have greater bactericidal action than the serum of the infected turkeys (Tables 7 and 8). Plasmae from the orally infected turkeys (Tables 9, 12, 14, 15, and 16) were more bactericidal than those of intravenously infected birds. The normal plasma was the best of the three when compared with the plasma of low agglutinating titer. When the agglutinating titer was sufficiently high, plasma of the orally infected turkeys showed

greater bactericidal action than the normal plasma.

Tables 17 and 18 show that the plasma of the turkeys which had been injected with dead S. pullorum antigen possessed better bactericidal action than the plasma of the birds which had been injected with living S. pullorum antigen.

Plasma lost its bactericidal property after absorption with S. pullorum antigen. The amount of bactericidal action lost depends on the amount of bacterial antigen used for the absorption. Plasma lost its bactericidal action also when a Salmonella choleraesuis antigen was used for absorption which shows non-specificity of bactericidal action. The results were obtained through many repeated experiments.

When plasma was diluted four times, the immune plasma showed better bactericidal action than the normal plasma. After 16 hours of incubation, the mixture of plasma and bacteria was pipetted out and plated in nutrient agar. The normal plasma showed numerous colonies in 1:16 dilutions, whereas the immune plasma showed numerous colonies in 1:64 dilutions. These two plasmae showed little or no growth of bacterial colonies at lower dilutions which indicates that the plasma is bactericidal or bacteristatic at the above dilutions.

In the presence of excess complement, normal plasma showed numerous colonies in 1:32 plasma (saline) dilutions whereas there were about the same number of colonies in the 1:128 immune plasma dilutions. This indicates that the bactericidal action increases some when sufficient complement is present.

An excess amount of guinea pig complement (0.1 ml) was added to each tube to see what influence it would have on bactericidal action. Table 19 shows that the bactericidal action improved slightly in the unheated normal and immune plasma, but it (guinea pig complement) had absolutely no influence on the plasma which had been heated at 56°C. for one hour. Heating at 56°C. may have destroyed the antibodies as well as the complement or guinea pig complement may not be an adequate substitute for turkey complement.

Agglutination tests. Ordinarily agglutination titration mixtures of plasma and antigen, incubated at 37°C., showed no prozones except sometimes with old plasma. No noticeable difference in agglutination or titer was observed when the pH ranged from 6 to 8.5. However, when incubated at 56°C., all the agglutinations at three different pH levels showed prozones in the first three dilutions and showed less inhibitory zone agglutination at a lower pH than at a higher pH.

When the immune plasma was heated at 56°C. for one hour, the precipitate filtered off, and the agglutination test was run at 56°C., the test showed no inhibitory zone at all (Table 20).

When the plasma was filtered through No. 03 Selas filters immediately after the blood was drawn from the birds, it showed an inhibitory zone as had the untreated plasma. If this plasma was allowed to stand for several days in the ice box and was then refiltered through a filter of the same porosity, the plasma showed little or no inhibitory zone. When plasma was frozen at -35°C. for two days and the sediment removed after thawing, it showed very little inhibitory zone.

When 0.1 ml. of fresh pullorum negative plasma was added to the immune plasma (0.1 ml.) which had been heated at 56°C. for one hour and filtered, it showed no inhibitory zone; but if the above immune plasma was mixed with 0.1 ml of old pullorum negative plasma, it showed some inhibitory zone (Table 21).

Table 22, shows results of absorption of plasma with S. pullorum and S. choleraesuis antigen. Both showed inhibitory zones before absorption but none afterward and both showed decrease in agglutinating titer after absorption. In one instance, plasma

which had been kept in an ice box (4°C.) for 6 days, and was then filtered, and subjected to an agglutinating test, showed no inhibitory zone.

All the results mentioned indicate that the inhibitory substance is in the colloidal state and that it can be removed from the plasma by various physical treatments such as aging, freezing, heating, centrifugation, and filtration. The plasma must be altered in some way before the inhibiting colloidal substance can be formed. Before going into further discussion, let us review the work of Shibley. He said that raising the temperature increases the quantity of agglutinin changed into agglutinoid. Now, if this is true, why is there no change of agglutinating titer after heating? In my experiments, the plasma which was heated at 56°C. for one hour showed a large amount of sediment after heating. When the sediment was removed it showed no decrease in agglutinating titer, which indicates that none of the agglutinin was lost after heating at 56°C. for one hour. In fact, in most cases it showed a stronger agglutination than the unheated plasma. So it is unlikely that it is due to the alteration of agglutinin. Another fact which shows that there is no alteration in the agglutinin is that the filtered heated plasma which gives no prozone at 56°C. incubation, will give prozone when old, normal plasma is added to it. We know that there is no pullorum

agglutinating antibody in normal plasma, yet the old, altered pullorum negative plasma causes prozone when added to the pullorum positive plasma which had been heated at 56°C. for one hour and centrifuged (Table 21). This suggests that a non-specific colloidal substance interferes with the agglutination. When plasma was raised to a higher pH, (8.5) a wider range of prozone was produced. At lower pH (5.4) little or no prozone was present. The above may have something to do with the iso-electric point of protein. Since pH 5.4 is almost the iso-electric point at which most of the globulin may be precipitated this pH may cause the flocculation of the colloidal particles, and thereby remove the substance which interferes with agglutination. The prozone can also be removed when a sufficient quantity of bacterial antigen may absorb most of the colloidal particles; therefore, no prozone will occur in the absorbed plasma (Jamil and Stafseth 1949). The above results are unlikely to be due to degraded agglutinin, since prozone "A" occurs without heating serum.

Plasma from infected birds shows larger colloidal particles present more often than the plasma from normal birds, and it has been found that the rate of sedimentation of infected blood is greater than that of normal blood. The cause of this phenomenon is not clear. It is apparently connected with the ratio of albumin, glo-

bulin, and fibrinogen in the plasma, which influences the formation of larger colloidal particles and the rate of sedimentation; or there may be some degree of protein alteration in the infected blood which causes the protein to become precipitated more readily than in normal blood. This may lead to an explanation of the reasons why diseased blood of pullorum infected turkeys is altered more easily than normal blood, as shown by the formation of larger colloidal particles in plasma after removal from the blood stream. Very old plasma which shows heavy precipitate does not give a prozone when the precipitate is filtered off before titrating agglutinating sera. It is usually the intermediate alteration of plasma that causes a prozone (Table 23). It appears that a certain degree of alteration of plasma protein produces the proper size of colloidal particles which are the cause of prozones. When the colloidal particles have gone beyond this size, the inhibitory effect is lost. It is possible that the very old plasma loses its inhibitory effect because the plasma-protein has been aggregated into larger particles which no longer can be adsorbed on the surface of the bacteria cell, and therefore, does not prevent the adsorption of agglutinating antibody.

The lower bactericidal action of the undiluted infected blood plasma may also be due to the

colloidal particles which are adsorbed on the surface of the bacterial cell preventing absorption of antibody. The fact that diluted infected blood plasma shows greater bactericidal action than normal diluted plasma, indicates the possibility that the diluted plasma does not have a sufficient quantity of colloidal particles to prevent the absorption of antibodies. Plasma from the orally infected turkeys with a high agglutinating titer showed greater bactericidal property than plasma of the intravenously infected turkeys. This may indicate that little or no protein alteration took place in the former whereas more protein alteration occurred in the latter or it may have to do with the contacts which the antigen makes with immunologically active tissues in the intestinal wall (Tables 10 and 12).

The results of this experiment indicate that plasma is apt to deteriorate after it has been removed from the circulatory system. During the process of deterioration, certain inhibitory colloidal particles are formed. These colloidal particles when adsorbed on the surface of bacterial cells, interfere with the absorption of antibody, thereby producing prozone. Since such colloidal protein particles appear to be formed by the alteration (denaturation) of protein after it has been drawn from the blood stream, the bactericidal action of the infected turkey plasma may be better <u>in vivo</u> than <u>in vitro</u>.

Summary

1. The average number of blood cells per cubic mm. of blood in normal turkeys was: 2,600,000 erythrocytes and 12,000 leucocytes. The percentage of the various types of leucocytes was:

heterophils	43%
lymphocytes	51%
eosinophils	1%
basophils	2%
monocytes	3%

2. The erythrocytes decreased after oral or intravenous administration of S. pullorum.

3. There was a sharp rise in heterophils after intravenous administration of S. pullorum.

4. Lymphocytes did not increase much during the first part of the infection, but gradually increased during the later stages of the infection.

5. The agglutinating titer rose before there was any significant increase in lymphocytes.

6. Plasma showed greater bactericidal property than serum.

7. Normal undiluted plasma showed greater bactericidal property than plasma from infected turkeys.

8. Normal plasma diluted to 1:16 showed less bactericidal action than plasma from the infected turkeys in the same dilution.

9. Plasma from the orally infected turkeys showed greater bactericidal power than plasma from the intravenously infected ones.

10. Plasma with a high agglutinating titer showed the best bactericidal action.

11. The bactericidal property was destroyed by heating at 56°C. for one hour.

12. When the immune plasma was heated at 56°C. for one hour, the precipitate filtered off, and the agglutination test showed no inhibitory zone. Freezing and aging the immune plasma will remove the factor which causes prozones.

13. Old, deteriorated normal plasma added to the prozone-free plasma caused prozone which suggests that the prozone is due to non-specific colloidal particles.

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Table 1

Dilutions of *Salmonella pullorum* Suspension Used in this Experiment

Tubes	1	2	3	4	5	6	7	8	9
Saline	9 ml	9 ml	9 ml	9 ml	9 ml	9 ml	9 ml	9 ml	9 ml
Pullorum Suspension	1 ml								
Amt. of Sal.pul. Susp. Transferred	1 ml →	→	→	→	→	→	→	→	
Dilutions	10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	10^{-6}	10^{-7}	10^{-8}	10^{-9}

Table 2

Agglutination Test Mixtures

	Dilutions									
Tubes	1	2	3	4	5	6	7	8	9	10
Dilutions	1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240
Antigen added	1.9ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml	1 ml
Plasma added	0.1ml									
Mixture transferred	→	1 ml →	1 ml →	1 ml →	1 ml →	1 ml →	1 ml →	1 ml →	1 ml →	1 ml

The Salmonella pullorum antigen was adjusted to the desired pH, (8.4) 1.9 ml. of the antigen was placed in tube No. 1 and 1 ml in the following tubes: 0.1 ml of the testing plasma was pipetted into tube No.1 and mixed well; then 1 ml was transferred into tube No. 2 etc.

Table 3

The Bactericidal Activities of 2 Normal Sera

Serum 1

Normal serum dilution	Number of bacteria added	Colony Count Incubation period in hours 4	8	24	48
1:2	7×10^9	N	N	29	0
1:2	7×10^8	N	338	2	0
1:2	7×10^7	698	144	1	0
1:2	7×10^6	84	21	0	0
1:2	7×10^5	16	7	0	0
1:2	7×10^4	9	2	0	0
1:2	7×10^3	2	0	0	0
1:2	7×10^2	4	0	0	0
1:2	7×10^1	0	0	0	0

Serum 2

1:2	7×10^9	N	N	31	0
1:2	7×10^8	N-	231	0	0
1:2	7×10^7	428	35	0	0
1:2	7×10^6	27	6	0	0
1:2	7×10^5	18	3	0	0
1:2	7×10^4	7	0	0	0
1:2	7×10^3	0	0	0	0
1:2	7×10^2	0	0	0	0
1:2	7×10^1	0	0	0	0

N = too numerous to count

Table 4

The Bactericidal Activities of 2 Normal Plasmae

Plasma 1

Normal plasma dilution	Number of bacteria added	Colony Count Incubation period in hours 4	8	24	48
1:2	8×10^9	N	N	N	N
1:2	8×10^8	N	N	N	N
1:2	8×10^7	N	N	1000	201
1:2	8×10^6	N	N-	493	29
1:2	8×10^5	634	491	7	0
1:2	8×10^4	131	110	1	0
1:2	8×10^3	36	25	0	0
1:2	8×10^2	15	3	0	0

Plasma 2

Normal plasma dilution	Number of bacteria added	Colony Count Incubation period in hours 4	8	24	48
1:2	8×10^9	N	N	N	N
1:2	8×10^8	N	N	N	N
1:2	8×10^7	N	N	952	338
1:2	8×10^6	N-	1144	247	35
1:2	8×10^5	630	358	49	3
1:2	8×10^4	138	93	6	0
1:2	8×10^3	38	18	0	0
1:2	8×10^2	12	4	0	0

N = Too numerous to count

Table 5

Rate of Bactericidal Action of Serum and Plasma

Normal Serum Dilution	Number of Bacteria added	Colony Count Incubation period in hours 4	8	24	48
1:2	15×10^9	N	N	N	N
1:2	15×10^8	N	N	208	N
1:2	15×10^7	N	N	35	0
1:2	15×10^6	1480	619	2	0
1:2	15×10^5	263	153	0	0
1:2	15×10^4	73	41	0	0
1:2	15×10^3	30	6	0	0
1:2	15×10^2	19	6	0	0

Normal Plasma Dilution	Number of Bacteria added	Colony count Incubation period in hours 4	8	24	48
1:2	15×10^9	N	N	18	0
1:2	15×10^8	1110	26	12	0
1:2	15×10^7	21	7	2	0
1:2	15×10^6	3	0	0	0
1:2	15×10^5	0	0	0	0
1:2	15×10^4	1	0	0	0
1:2	15×10^3	0	0	0	0
1:2	15×10^2	0	0	0	0

Table 6

Rate of Bactericidal Action of Serum and Plasma

Normal serum dilution	Number of bacteria added	Colony Count Incubation period in hours 4	8	24	48
1:2	15×10^9	N	782	19	1
1:2	15×10^8	N	446	1	0
1:2	15×10^7	684	64	0	0
1:2	15×10^6	93	8	0	0
1:2	15×10^5	9	1	0	0
1:2	15×10^4	2	0	0	0
1:2	15×10^3	3	0	0	0
1:2	15×10^2	1	0	0	0

Normal plasma dilution	Number of bacteria added	Colony Count Incubation period in hours 4	8	24	48
1:2	15×10^9	N	414	18	4
1:2	15×10^8	1254	47	3	0
1:2	15×10^7	322	19	0	0
1:2	15×10^6	39	2	2	0
1:2	15×10^5	5	0	0	0
1:2	15×10^4	1	0	0	0
1:2	15×10^3	1	0	0	0
1:2	15×10^2	1	0	0	0

Table 7

Rate of Bactericidal Action of Serum and Plasma

From Infected Turkey

Infected serum dilution	Number of bacteria added	Colony Count Incubation period in hours 4	8	24	48
1:2	7×10^9	N	N	N	N
1:2	7×10^8	N	N	N	181
1:2	7×10^7	N	N	N-	4
1:2	7×10^6	1522	480	223	0
1:2	7×10^5	164	182	47	0
1:2	7×10^4	76	27	2	0
1:2	7×10^3	17	20	1	0
1:2	7×10^2	18	21	1	0

Infected plasma dilution	Number of bacteria added	Colony Count Incubation period in hours 4	8	24	48
1:2	7×10^9	N	N	N	N
1:2	7×10^8	N	N	N	N
1:2	7×10^7	N	N	N-	682
1:2	7×10^6	890	464	219	6
1:2	7×10^5	62	75	5	2
1:2	7×10^4	34	14	1	1
1:2	7×10^3	13	9	0	0
1:2	7×10^2	8	2	0	0

N = too numerous to count

Table 8

Rate of Bactericidal Action of Serum and Plasma From Infected Turkey

Infected serum dilution	Number of bacteria added	Colony Count Incubation period in hours 4	8	24	48
1:2	6×10^9	N	882	8	1
1:2	6×10^8	1750	584	3	0
1:2	6×10^7	207	39	0	0
1:2	6×10^6	29	4	0	0
1:2	6×10^5	2	0	0	0
1:2	6×10^4	1	0	0	0
1:2	6×10^3	1	0	0	0
1:2	6×10^2	0	0	0	0
1:2	6×10^1	0	0	0	0

Infected plasma dilution	Number of bacteria added	Colony Count Incubation period in hours 4	8	24	48
1:2	6×10^9	N	336	0	0
1:2	6×10^8	1044	57	0	0
1:2	6×10^7	44	3	0	0
1:2	6×10^6	7	1	0	0
1:2	6×10^5	2	0	0	0
1:2	6×10^4	0	0	0	0
1:2	6×10^3	0	0	0	0
1:2	6×10^2	0	0	0	0
1:2	6×10^1	0	0	0	0

Table 9

Rate of Bactericidal Action of Plasma

Orally Infected Turkey

	No. of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma diluted to 1:2	8×10^9	N	N	912	49
	8×10^8	N	N	121	9
	8×10^7	N-	N	59	3
	8×10^6	1221	305	0	1
	8×10^5	304	84	0	0
	8×10^4	105	0	0	0
	8×10^3	50	0	0	0

Intravenously Infected Turkey

	Number of bacteria added	Colony count Period of incubation in hours 4	8	24	48
Plasma diluted to 1:2	8×10^9	N	N	N	N
	8×10^8	N	N	382	23
	8×10^7	N	N	52	1
	8×10^6	1339	422	8	1
	8×10^5	435	109	0	2
	8×10^4	176	48	0	0
	8×10^3	117	39	0	0

Table 10

Rate of Bactericidal Action of Plasma

Orally Infected Turkey

	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma diluted to 1:2	8×10^9	N	N-	524	14
	8×10^8	N	1868	12	2
	8×10^7	N	411	5	1
	8×10^6	278	102	2	0
	8×10^5	58	9	0	0
	8×10^4	15	0	0	0
	8×10^3	4	0	0	0

Intravenously Infected Turkey

	Number of bacteria added	Colony count Period of incubation in hours 4	8	24	48
Plasma diluted to 1:2	8×10^9	N	N	N	N
	8×10^8	N	N	N	N
	8×10^7	N	N	N	N
	8×10^6	1460	1208	N	N
	8×10^5	336	254	N	N
	8×10^4	86	54	N	N
	8×10^3	57	48	N	N

Table 11

Rate of Bactericidal Action of Plasma

Both Turkeys Were Infected Intravenously

	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma diluted to 1:2	8×10^9	N	N	258	
	8×10^8	N	N	107	
	8×10^7	N-	1360	15	
	8×10^6	332	281	0	
	8×10^5	108	42	0	

Agglutinating Titer = 1/640

	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma diluted to 1:2	8×10^9	N	N	N	
	8×10^8	N	N	N	
	8×10^7	N-	1400	1380	
	8×10^6	437	310	36	
	8×10^5	120	50	0	

Agglutinating Titer = 1/320

Table 12

Rate of Bactericidal Action of Plasma

Orally Infected Turkey

	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma dilution 1:2	8×10^9	N	N	242	
	8×10^8	N	N	11	
	8×10^7	N	761	0	
	8×10^6	820	51	0	
	8×10^5	288	22	0	
	8×10^4	21	3	0	
	8×10^3	11	0	0	

Intravenously Infected Turkey

	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma dilution 1:2	8×10^9	N	N	960	
	8×10^8	N	N	74	
	8×10^7	N	N	21	
	8×10^6	N	221	22	
	8×10^5	1064	141	0	
	8×10^4	373	40	0	
	8×10^3	73	4	0	

Table 13

Rate of Bactericidal Action of Plasma

Plasma of an orally infected turkey

	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma dilution 1:2	8×10^9	N	1190	13	
	8×10^8	N-	824	3	
	8×10^7	504	95	1	
	8×10^6	11	7	0	
	8×10^5	4	0	0	
	8×10^4	2	0	0	
	8×10^3	1	0	0	

Plasma of an intravenously infected turkey

	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma dilution 1:2	8×10^9	N	N	N	
	8×10^8	N	N	N	
	8×10^7	N	N	N	
	8×10^6	760	712	N	
	8×10^5	213	161	N	
	8×10^4	51	54	N	
	8×10^3	18	13	N	

Table 14

Rate of Bactericidal Action of Plasma

Plasma of an orally infected turkey with a high agglutinating titer.

Reacting substance	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma dilution 1:2	9×10^9	N	N	N	N
	9×10^8	N	N	N-	95
	9×10^7	N	814	536	1
	9×10^6	N-	92	11	0
	9×10^5	420	35	0	0
	9×10^4	106	12	0	0
	9×10^3	67	3	0	0

Plasma of an intravenously infected turkey.

Plasma dilution 1:2	9×10^9	N	N	N	N
	9×10^8	N	N	N	N
	9×10^7	N	N	N-	776
	9×10^6	N	N-	1426	115
	9×10^5	N-	442	256	55
	9×10^4	344	162	112	11
	9×10^3	140	92	52	8

Plasma of a normal turkey.

Plasma dilution 1:2	9×10^9	N	N	N	N
	9×10^8	N	N	N	N
	9×10^7	N	N	N	N
	9×10^6	N	N	N	N
	9×10^5	822	N	N	N
	9×10^4	235	914	N	N
	9×10^3	111	141	N	N

Table 15

Rate of Bactericidal Action of Plasma

Plasma from an orally infected turkey with a high agglutinating titer.

Reacting substance	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma dilution 1:2	6×10^9	N	N	N	
	6×10^8	N	N	241	
	6×10^7	N	N	3	
	6×10^6	N-	472	0	
	6×10^5	382	101	0	
	6×10^4	66	5	0	
	6×10^3	20	0	0	

Plasma from an intravenously infected turkey with a high agglutinating titer.

Reacting substance	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma dilution 1:2	6×10^9	N	N	N	
	6×10^8	N	N	N	
	6×10^7	N	N	N	
	6×10^6	N-	451	360	
	6×10^5	528	301	61	
	6×10^4	61	6	8	

Plasma from a normal turkey

Reacting substance	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma dilution 1:2	6×10^9	N	N	N	
	6×10^8	N	N	N	
	6×10^7	N	N	N	
	6×10^6	N	N	N	
	6×10^5	360	801	N	
	6×10^4	70	147	N	

Table 16

Rate of Bactericidal Action of Plasma

Plasma from an orally infected turkey with a high agglutinating titer.

Reacting substance	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma dilution 1:2	6×10^9	N	N	N	
	6×10^8	1101	1022	242	
	6×10^7	295	101	2	
	6×10^6	47	21	0	
	6×10^5	6	5	0	
	6×10^4	3	0	0	
	6×10^3	1	0	0	

Plasma from an intravenously infected turkey with a high agglutinating titer.

Reacting substance	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma dilution 1:2	6×10^9	N	N	N	
	6×10^8	N	N	N-	
	6×10^7	N	310	6	
	6×10^6	1110	550	1	
	6×10^5	207	102	0	
	6×10^4	63	7	0	
	6×10^3	5	1	0	

Plasma from a normal turkey

Reacting substance	Number of bacteria added	Colony Count Period of incubation in hours 4	8	24	48
Plasma dilution 1:2	6×10^9	N	N	N	
	6×10^8	N	N	N	
	6×10^7	N	N	N	
	6×10^6	891	N	N	
	6×10^5	431	1120	N	
	6×10^4	71	220	N	
	6×10^3	9	26	N	

Table 17

Rate of Bactericidal Action of Plasma

Tube No.	Dilution of plasma	Number of bacteria added	Colony count 4 hour incubation ID	IDA	I	IA	N'
1	1:2	15×10^6	N	N	N	N	N
2	1:2	15×10^5	N	N	N	N	N
3	1:2	15×10^4	175	N	N	N	92
4	1:2	15×10^3	33	869	675	1007	19
5	1:2	15×10^2	7	123	87	102	2
			20 hour incubation				
1	1:2	15×10^6	10	N	N-	N	9
2	1:2	15×10^5	4	N	226	N	6
3	1:2	15×10^4	3	N	25	N	4
4	1:2	15×10^3	2	N-	13	188	0
5	1:2	15×10^2	2	215	2	115	0

ID = Plasma of a turkey immunized with dead S. pullorum.
IDA = Plasma of "ID" adsorbed with S. pullorum.
I = Plasma of a turkey immunized with live S. pullorum.
IA = Plasma of "I" adsorbed with S. pullorum.
N' = Plasma of a normal turkey.

Table 18

Rate of Bactericidal Action of Plasma

5 hour incubation

Tube No.	Dilution of plasma	Number of bacteria added	Colony Count N'	ID	I
1	1:2	7×10^7	N	N	N
2	1:2	7×10^6	N	N	N
3	1:2	7×10^5	N-	N	N
4	1:2	7×10^4	6	840	N
5	1:2	7×10^3	5	120	N
6	1:2	7×10^2	0	16	521

24 hour incubation

1	1:2	7×10^7	170	N	N
2	1:2	7×10^6	0	140	N
3	1:2	7×10^5	0	25	102
4	1:2	7×10^4	0	0	95
5	1:2	7×10^3	0	0	3
6	1:2	7×10^2	0	0	0

N' = Normal plasma
ID = Plasma of the infected turkey immunized with dead S. pullorum organisms.
I = Plasma of the infected turkey immunized with live S. pullorum organisms.

Table 19

Rate of Bactericidal Action of Plasma

Tube No.	Dilution of plasma	Number of bacteria added	N'	I	N'C	IC	NHC	IHC
1	1:4	7×10^3	74	12	16	2	N	N
2	1:8	7×10^3	790	95	64	12	N	N
3	1:16	7×10^3	N	186	360	45	N	N
4	1:32	7×10^3	N	N	N	120	N	N
5	1:64	7×10^3	N	N	N	1500	N	N
6	1:128	7×10^3	N	N	N	N	N	N
7	1:256	7×10^3	N	N	N	N	N	N
8	1:512	7×10^3	N	N	N	N	N	N
			16 hour incubation					
1	1:4	7×10^3	0	0	0	0	N	N
2	1:8	7×10^3	154	0	3	0	N	N
3	1:16	7×10^3	N	4	320	0	N	N
4	1:32	7×10^3	N	998	N	0	N	N
5	1:64	7×10^3	N	N	N	701	N	N
6	1:128	7×10^3	N	N	N	N	N	N
7	1:256	7×10^3	N	N	N	N	N	N
8	1:512	7×10^3	N	N	N	N	N	N

N' = Normal plasma
I = Plasma of the infected turkey
N'C = Normal plasma with excess complement added
IC = Plasma of the infected turkey with excess complement added.
N'HC = Normal plasma heated at 56°C. for one hour. with excess complement added.
IHC = Plasma of the infected turkey heated to 56°C. for 1 hour with excess complement added.

Table 20

Agglutination Titrations

	pH	Incubation temp.	Dilutions 1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240
A	6	37°C.	++±	+++	+++	+++	+++	+++	+++	++	+±	+
B	7	37°C.	++±	+++	+++	+++	+++	+++	+++	++	++	+
C	8.5	37°C.	++±	+++	+++	+++	+++	+++	+++	++	++	+
D	6	56°C.	+±	++	++	++±	+++	+++	+++	++±	++	+
E	7	56°C.	+	+±	++	+++	+++	+++	+++	+++	++	+
F	8.5	56°C.	+	+±	++	++±	+++	+++	+++	++±	++	+
G	6	56°C.	+++	+++	+++	+++	+++	+++	+++	++	+±	+
H	6	56°C.	+++	+++	+++	+++	+++	+++	+++	++	+±	+
I	6	56°C.	++±	++±	+++	+++	+++	+++	+++	++	+±	+

A)
B) = Plasma of different pH with 37°C. incubation
C)

D)
E) = Plasma of different pH with 56°C. incubation
F)

G = Plasma heated at 56°C. for 1 hour then filtered before running the agglutination test.

H = Plasma heated as in "G" with 0.1 ml. of egg albumin added to it.

I = Plasma heated as in "G" with 0.1 ml. of normal plasma added to it.

Table 21

Agglutination Titrations

	pH	Incubation temp.	Dilutions									
			1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240
U	8.4	56°C.	±	+	++	++	+++	+++	+++	++	+	-
V	8.4	56°C.	++±	+++	+++	+++	+++	+++	+++	++	+	-
W	8.4	56°C.	+++±	+++	+++	+++	+++	+++	+++-	++	±	-
X	8.4	56°C.	++	++	+++	+++	+++	+++	+++	++	+	-

U = Plasma filtered through Selas filter #03 immediately after blood was drawn from turkey.

V = Plasma which was refiltered through Selas filter #03 after it has been standing in ice box for 2 days.

W = Plasma heated at 56°C. for an hour, centrifuged and the supernatant used.

X = The supernatant of "W" plus 0.1 ml. of old stored normal plasma.

Table 22

Agglutination Titrations

	pH	Incubation temp.	Dilutions									
			1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240
P	8.4	37°C.	+±	++	+++	+++	+++	+++	+++	++	+±	+
Q	8.4	37°C.		+++±	+++	+++	+++	+++	+++	++	+	-
R	8.4	37°C.		++++	+++	+++	+++	+++	++±	++	+	-
S	8.4	37°C.		++++	++++	++++	+++	+++	+++	++±	++	+
T	8.4	37°C.		+++	+++	+++	+++	+++	+++	++±	++	+

P = Plasma untreated.

Q = Plasma absorbed with S. pullorum antigen.

R = Plasma absorbed with S. choleraesuis antigen.

S = Plasma left standing in ice box (4°C) for 6 days before tested.

T = Fresh plasma not stored.

Table 23

Agglutination Titrations

	pH	Temperature	Dilutions									
			1/20	1/40	1/80	1/160	1/320	1/640	1/1280	1/2560	1/5120	1/10240
J	8.4	56°C.	±	+	++	++±	+++	++	+	±	-	
K	8.4	56°C.	+++	+++	+++	+++	+++	++	+	±	-	
L	8.4	56°C.	+++±	+++±	+++	+++	+++	++	+	±	-	
M	8.4	56°C.	+	+	++	++	+	+	-			
N	8.4	56°C.	+++	+++	+++	+++	+	+	-			
O	8.4	56°C.	+	+	++	++±	+	+	-			

J = Plasma frozen for 3 days at -35°C. (not filtered).
K = Plasma of J, but filtered before testing.
L = Plasma heated at 56°C for 1 hour and filtered before testing.
M = Plasma not heated nor filtered.
N = Plasma heated to 56°C. for 1 hour then centrifuged, supernatant used.
O = Plasma heated as in N, but not centrifuged nor filtered.

Figure 1

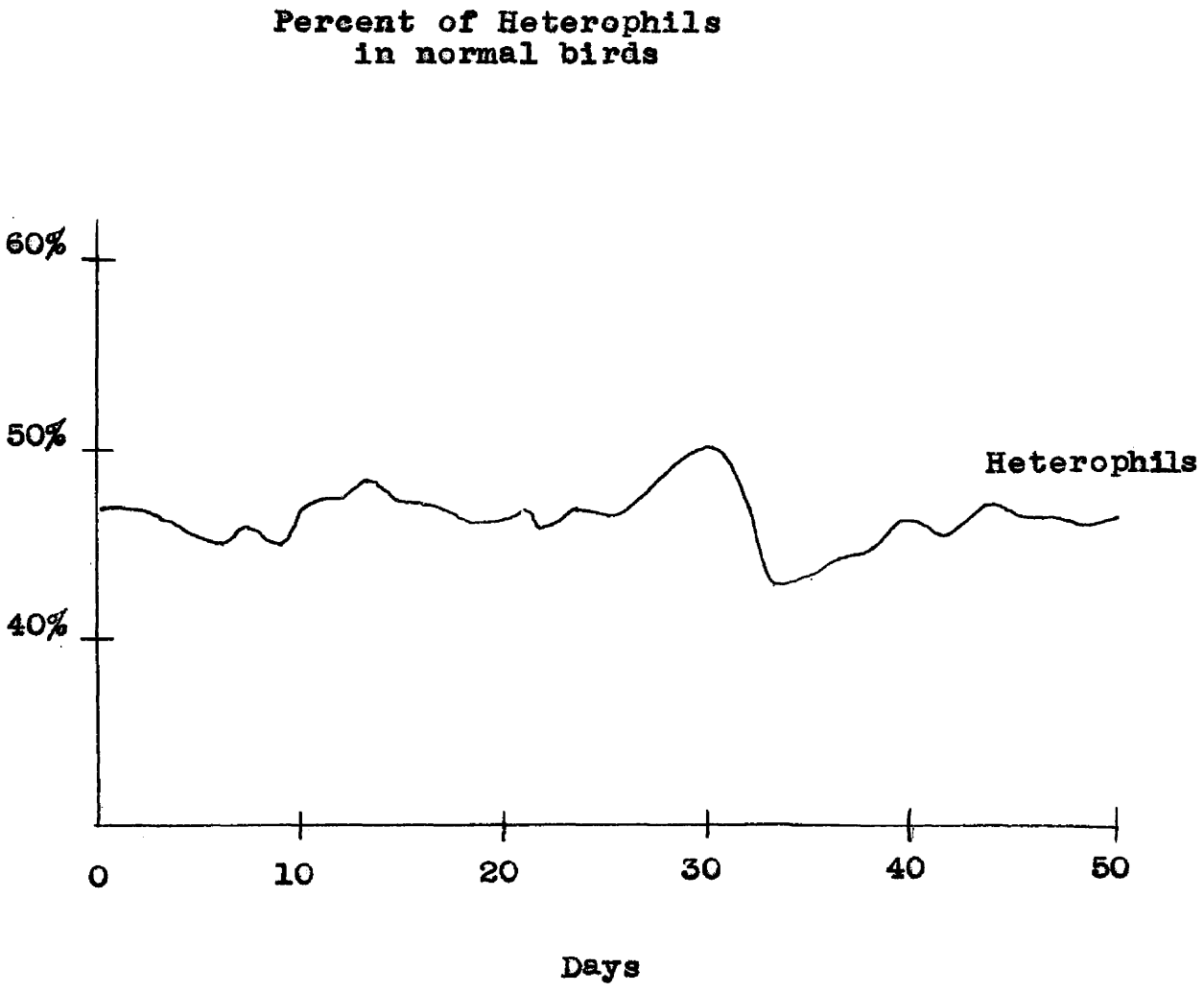

Percent of Heterophils
in normal birds
60%
50%
40%
Heterophils
0
10
20
30
40
50
Days

Figure 2

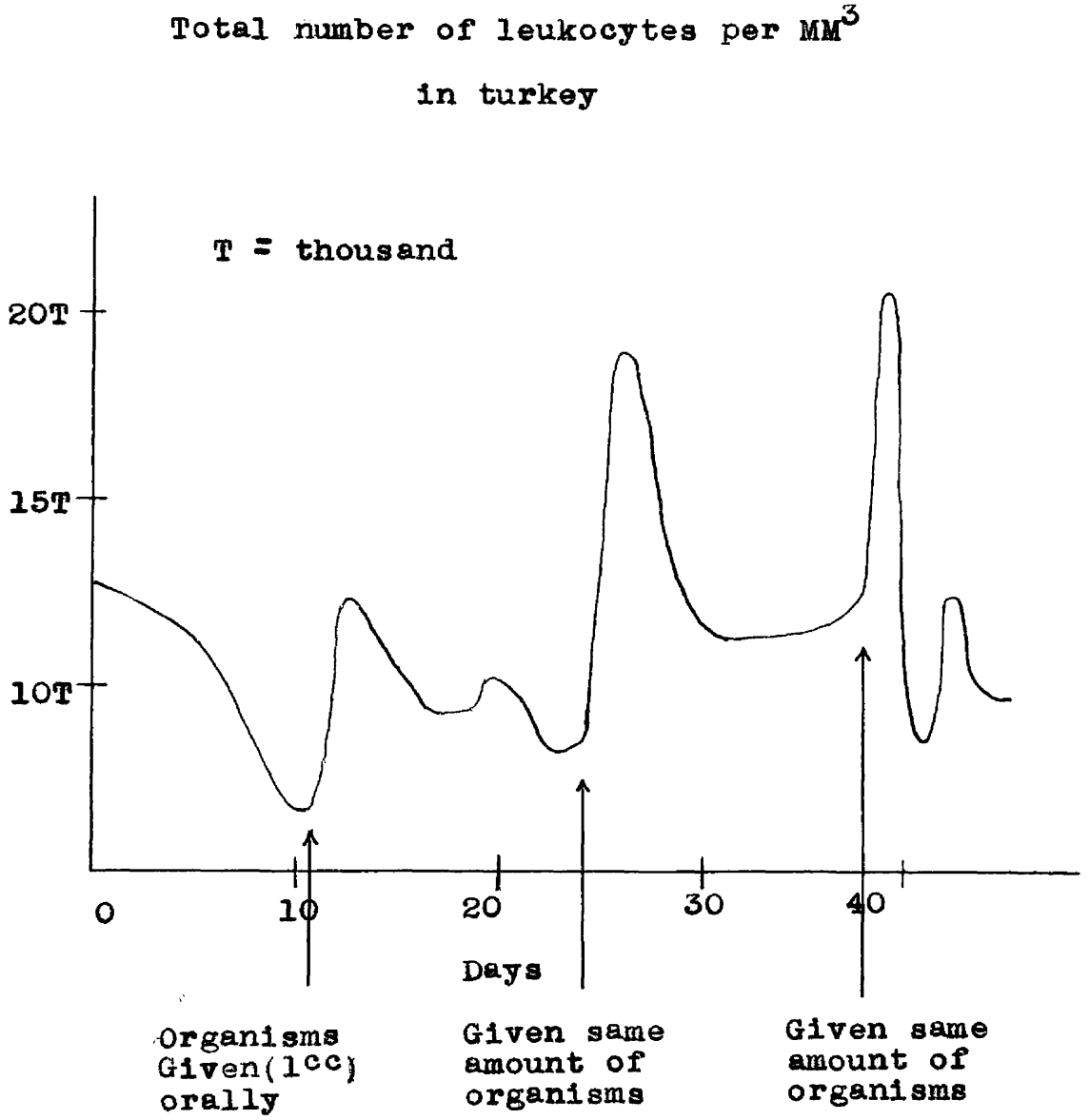

Total number of leukocytes per MM3
in turkey
T = thousand
20T
15T
10T
0
10
20
30
40
Days
Organisms Given(1cc) orally
Given same amount of organisms
Given same amount of organisms

Figure 3

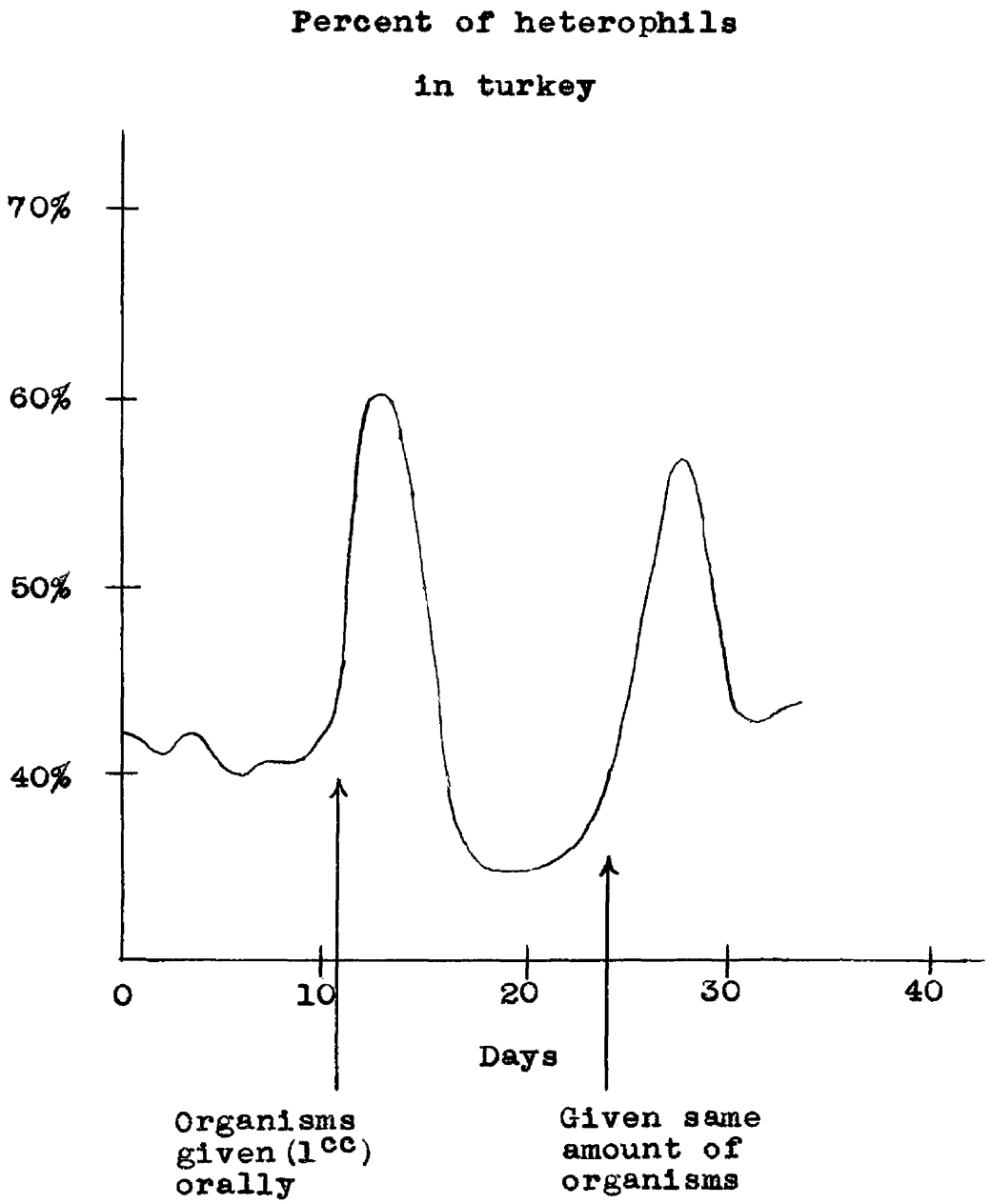

Percent of heterophils
in turkey
70%
60%
50%
40%
0
10
20
30
40
Days
Organisms given (1cc) orally
Given same amount of organisms

Figure 4

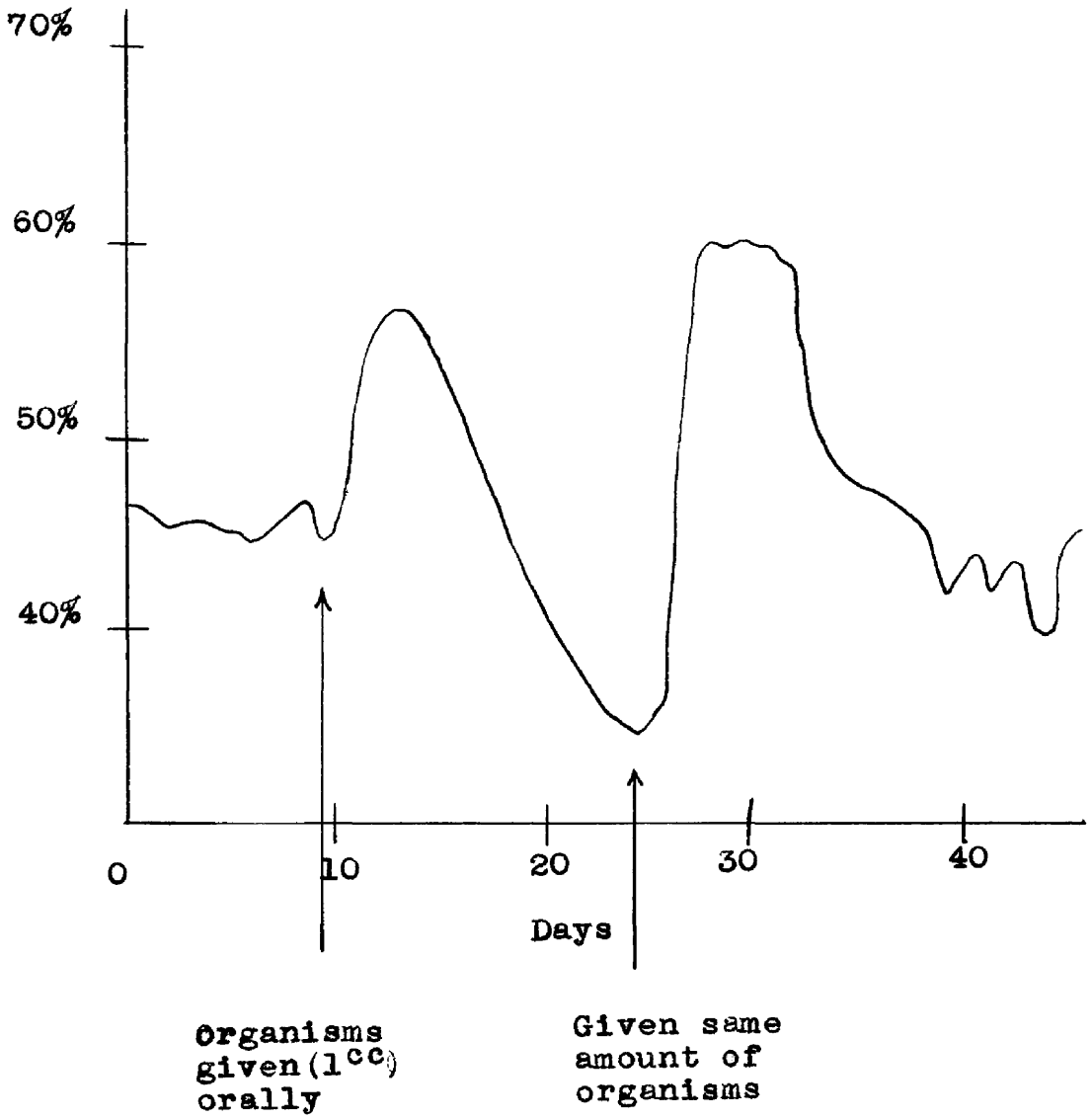

Percent of heterophils
in turkey
70%
60%
50%
40%
0
10
20
30
40
Days
Organisms
given (1cc)
orally
Given same
amount of
organisms

Figure 5

Number of red blood cells per cubic mm
in turkeys

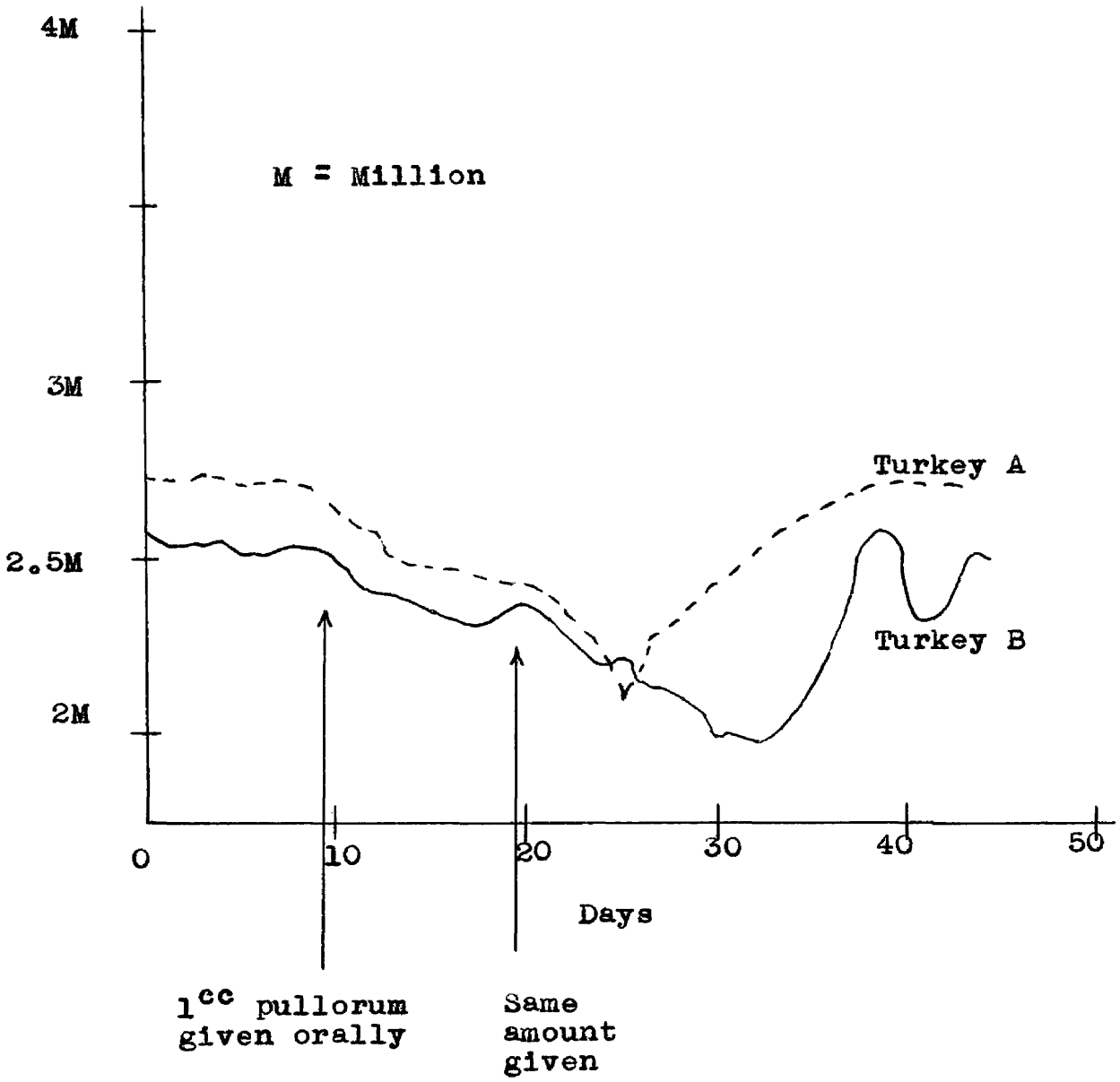


Figure 6

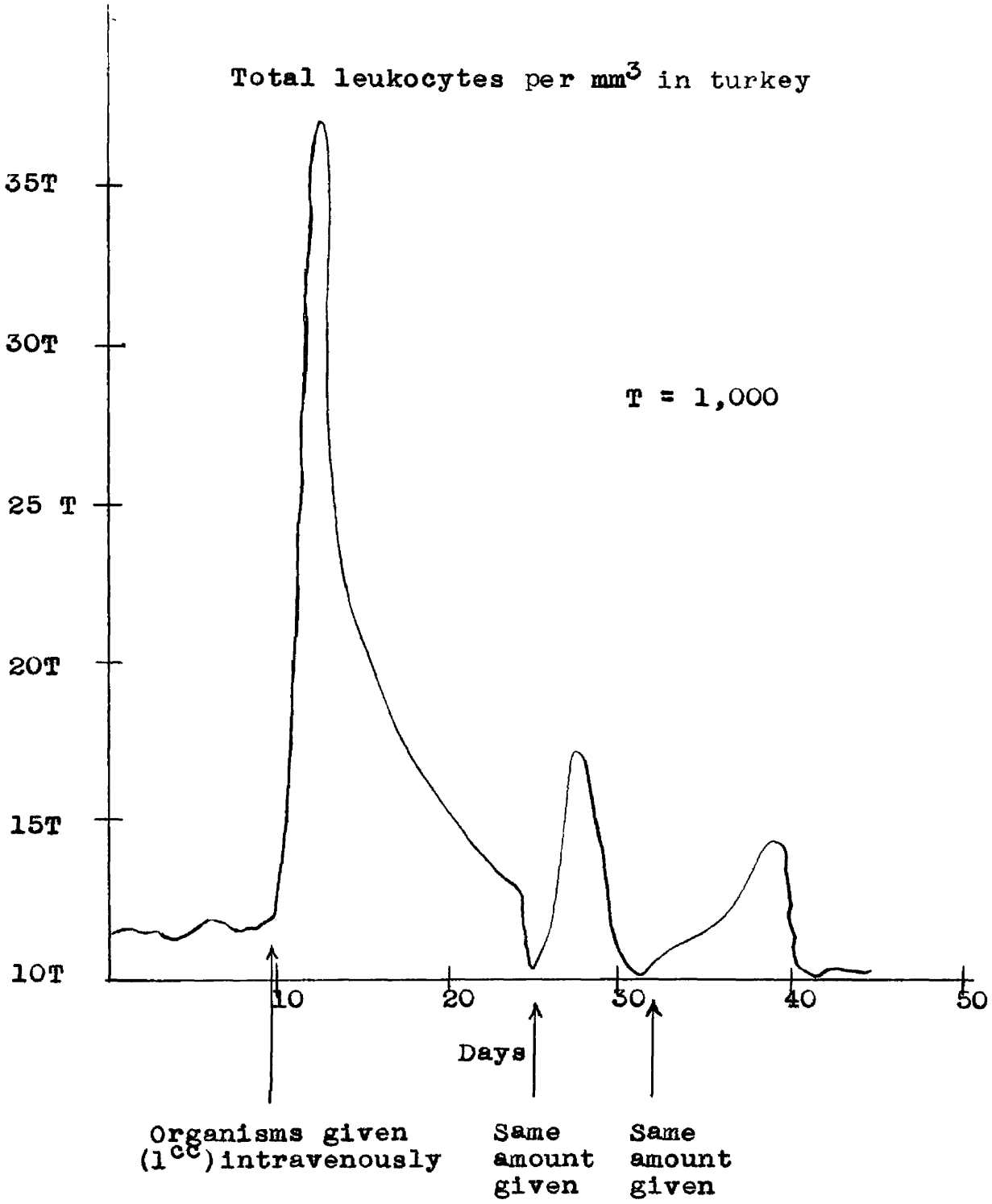

Total leukocytes per mm3 in turkey
35T
30T
25 T
20T
15T
10T
T = 1,000
10
20
30
40
50
Days
Organisms given (1cc) intravenously
Same amount given
Same amount given

Figure 7

Percent of heterophils
in turkey

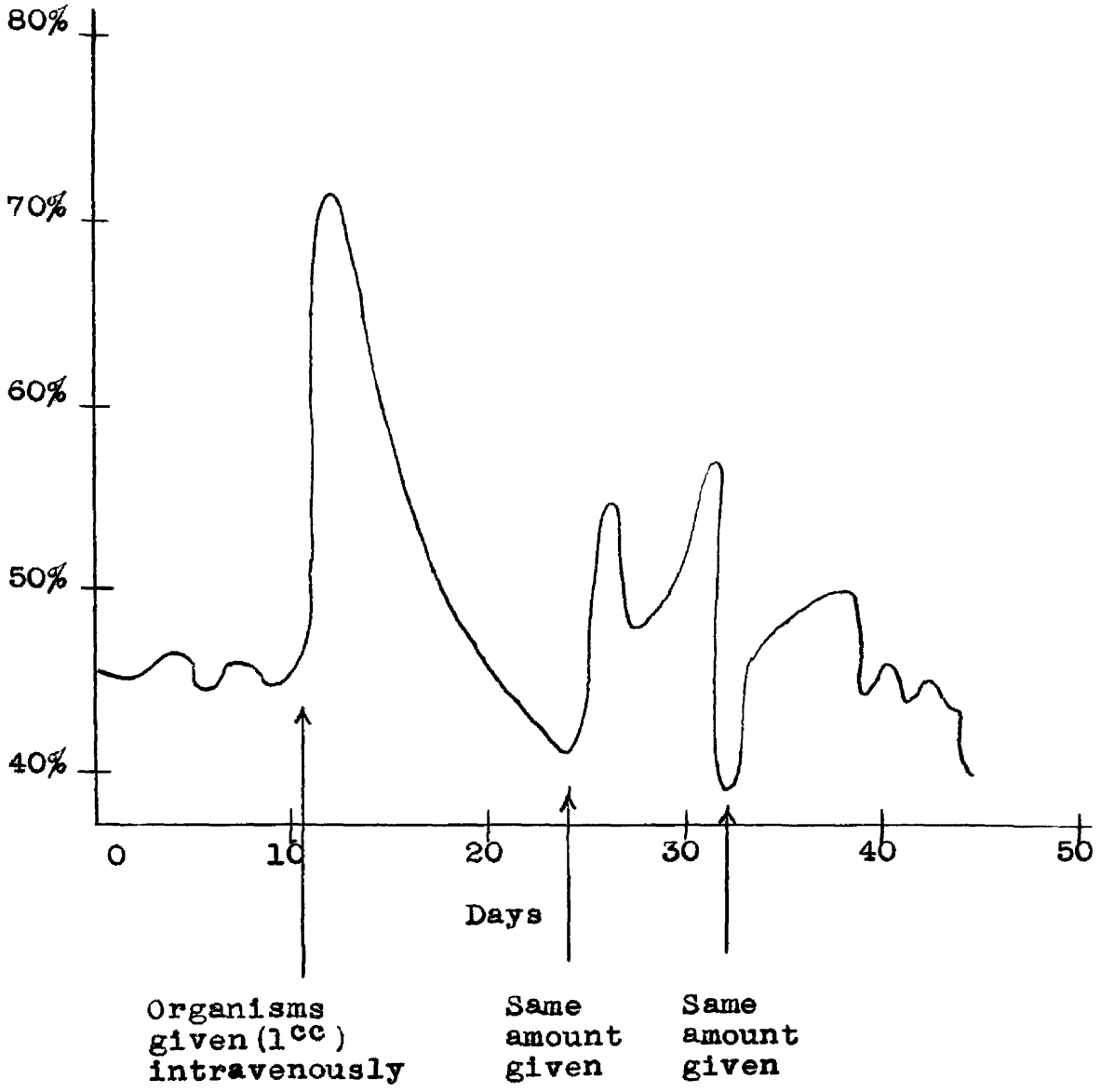


Figure 8

Number of red blood cells per cubic mm.
in turkey

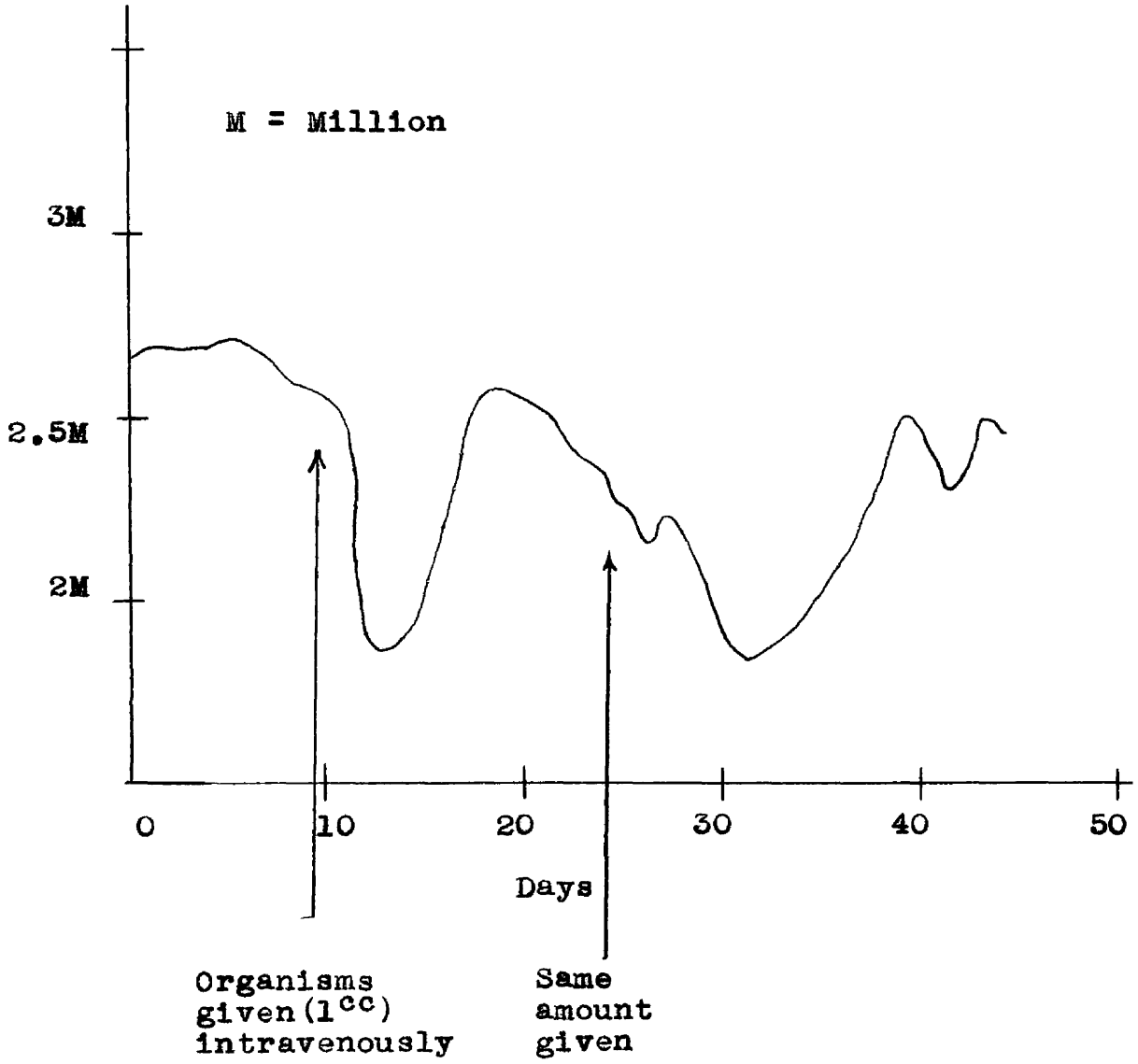


Figure 9

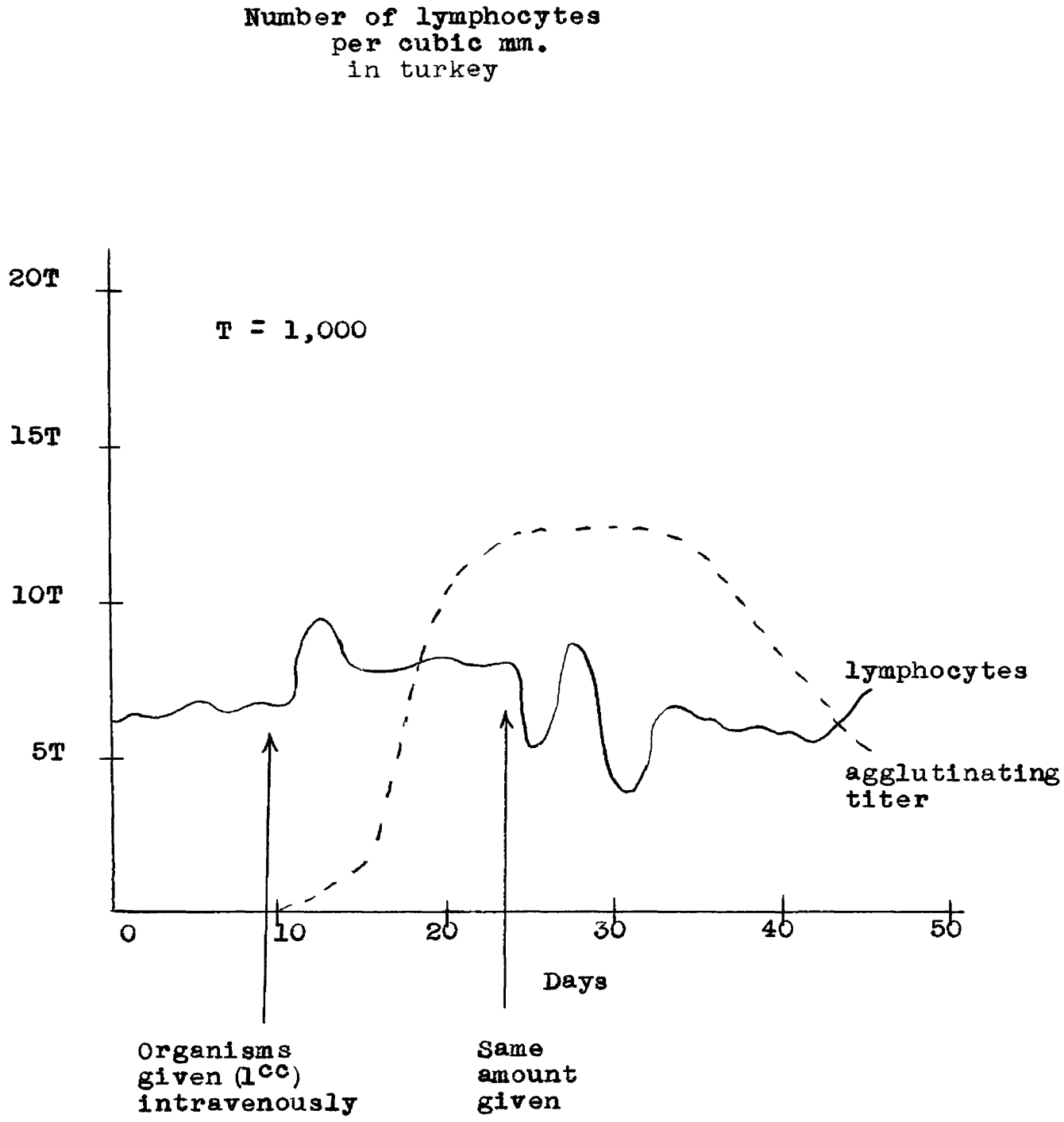

Number of lymphocytes
per cubic mm.
in turkey
20T
T = 1,000
15T
10T
5T
lymphocytes
agglutinating
titer
0
10
20
30
40
50
Days
Organisms
given (1cc)
intravenously
Same
amount
given

Figure 10

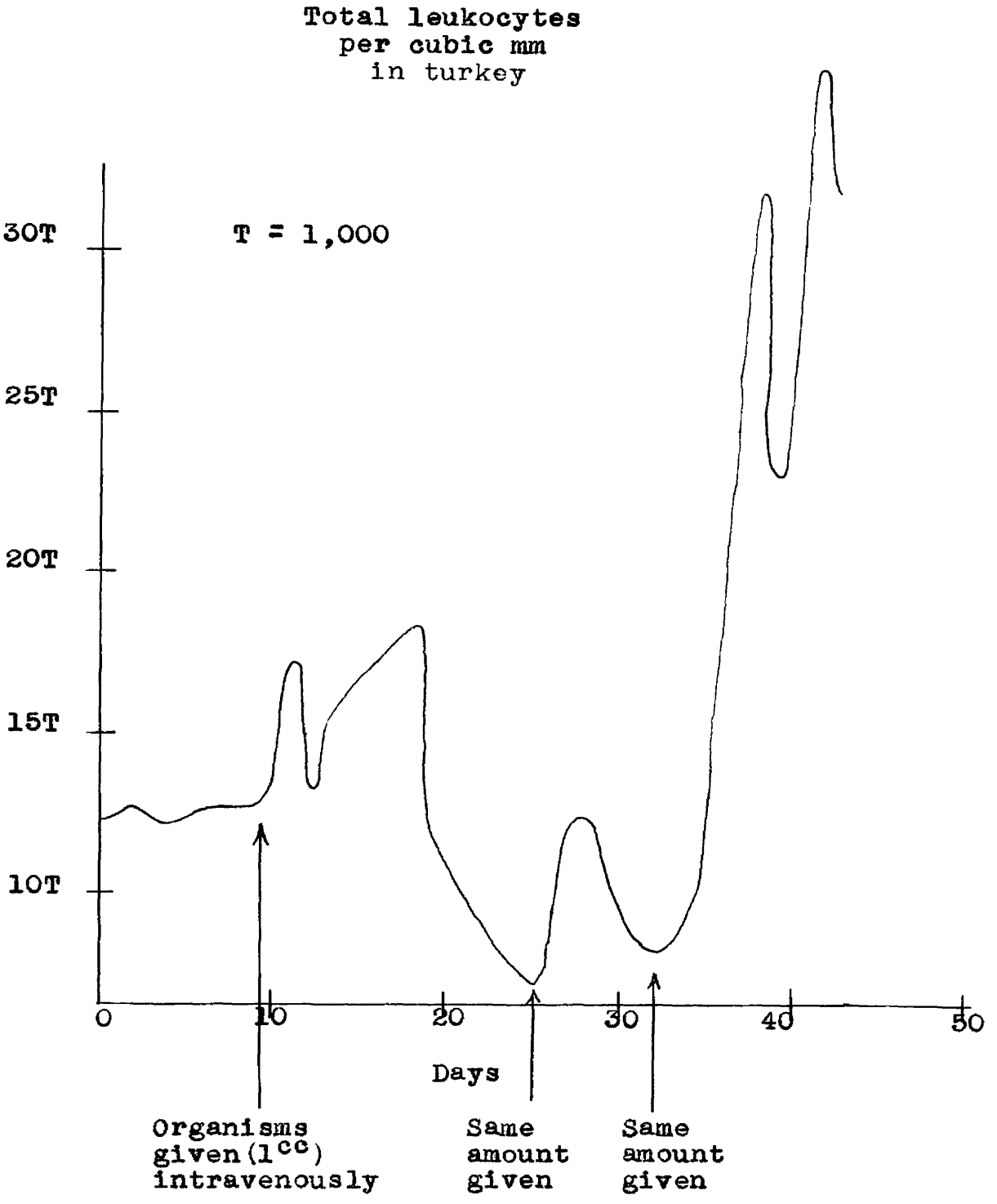

Total leukocytes
per cubic mm
in turkey
30T
T = 1,000
25T
20T
15T
10T
0
10
20
30
40
50
Days
Organisms
given (1 cc)
intravenously
Same
amount
given
Same
amount
given

Figure 11

Percent of heterophils
in turkey

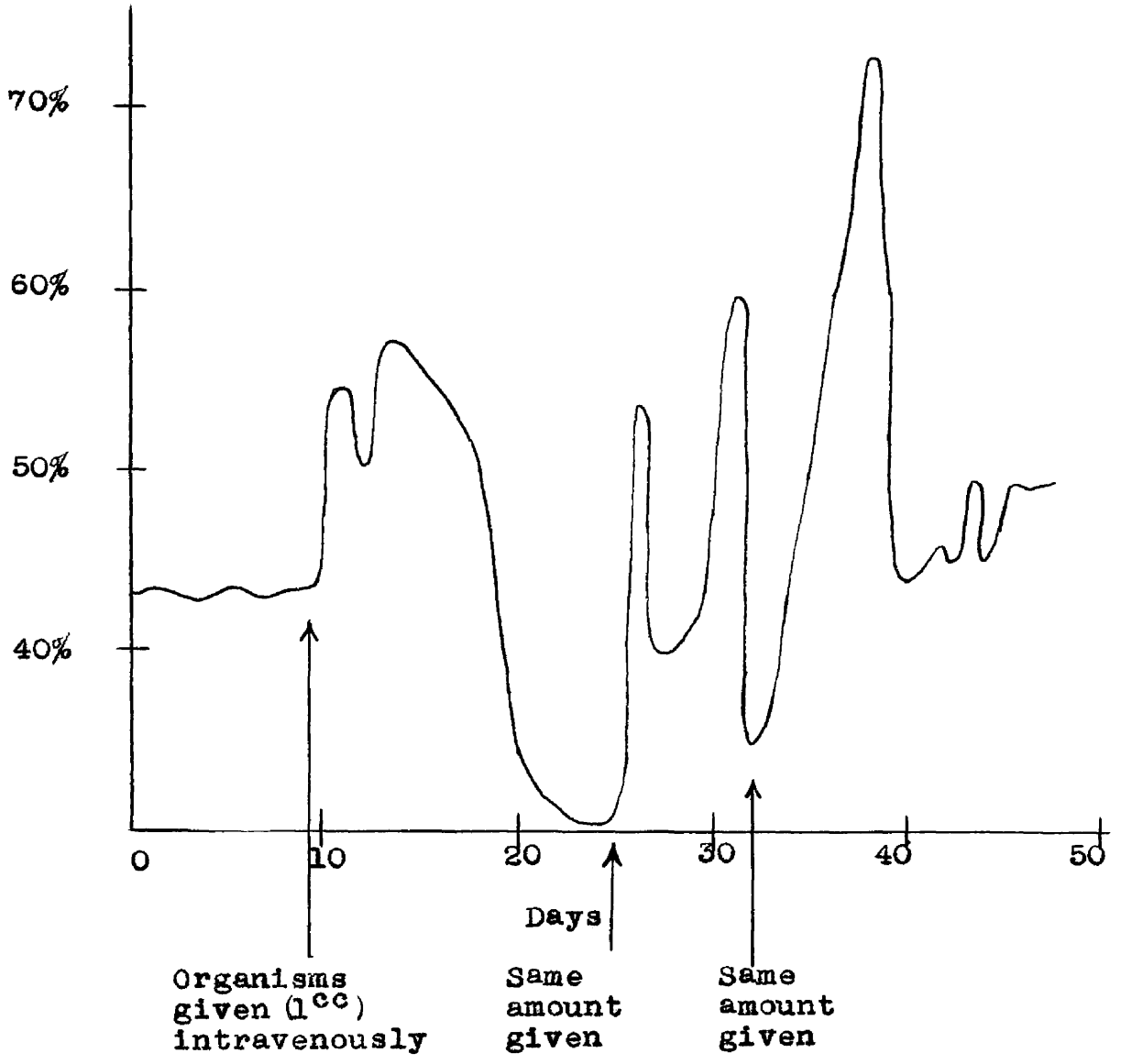


Figure 12

Number of red blood cells
per cubic mm.
in turkey

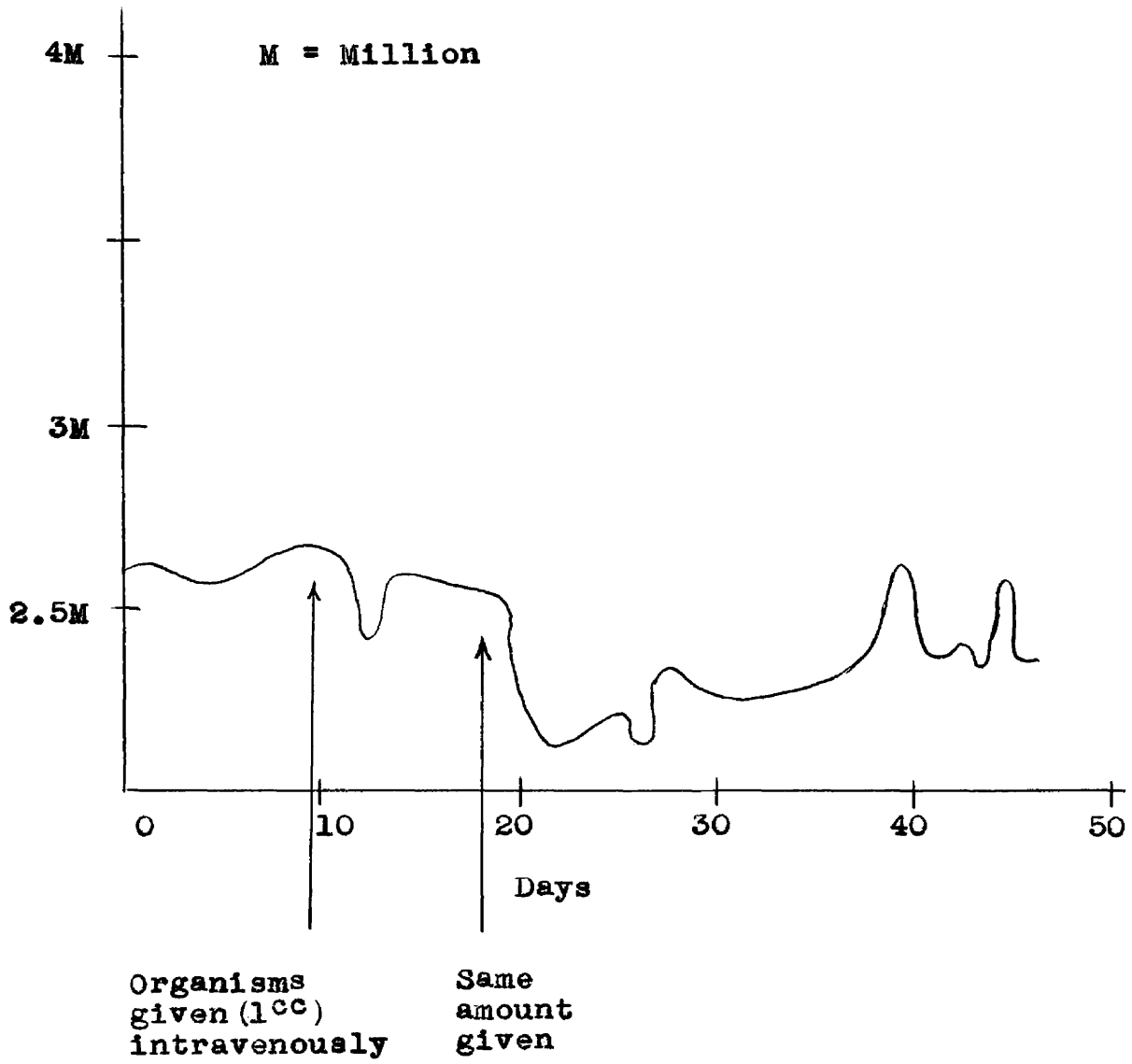


Figure 13

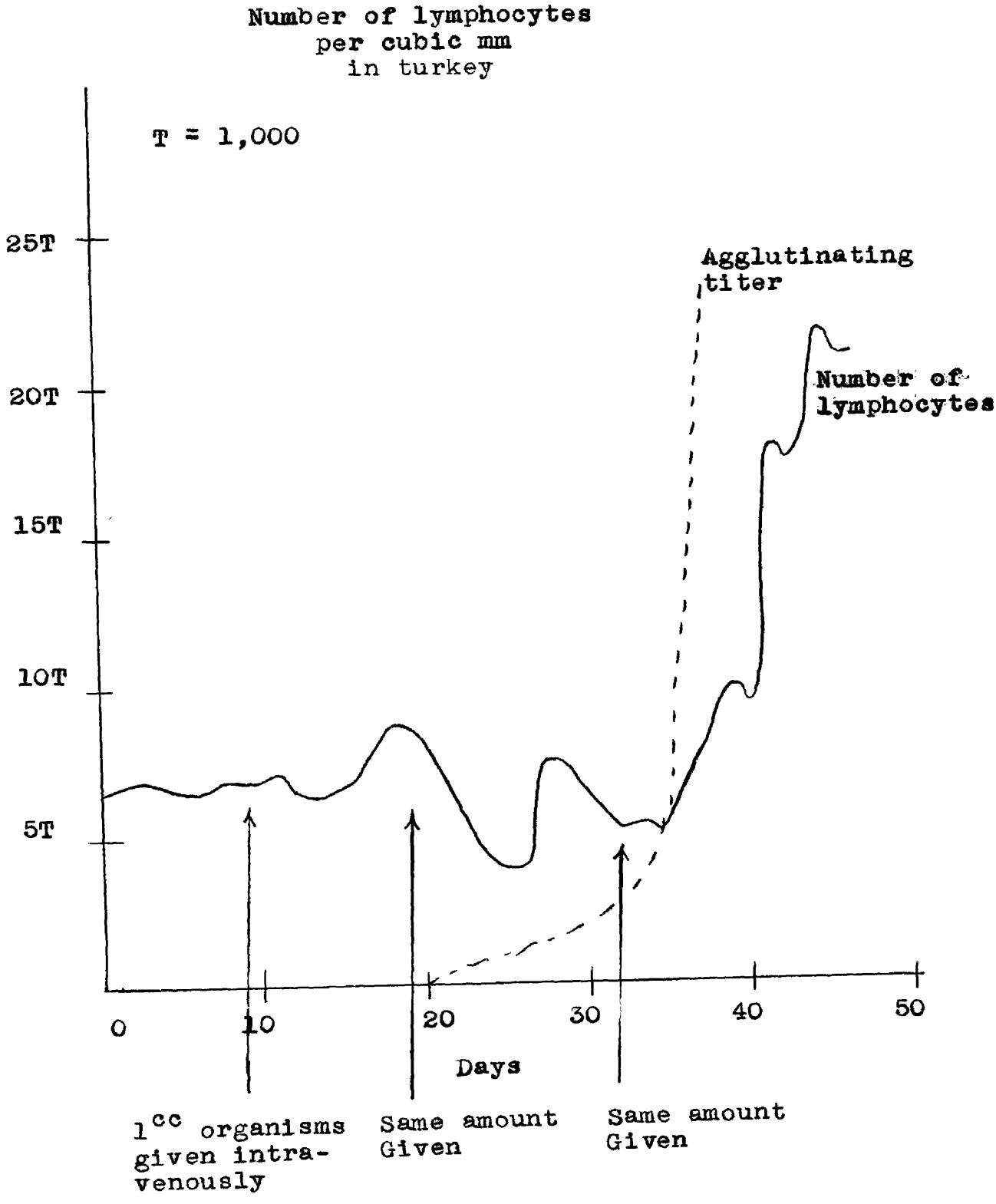

Number of lymphocytes
per cubic mm
in turkey
T = 1,000
25T
20T
15T
10T
5T
Agglutinating
titer
Number of
lymphocytes
0
10
20
30
40
50
Days
1cc organisms
given intra-
venously
Same amount
Given
Same amount
Given

Figure 14

Total leukocytes
per cubic mm
in turkey

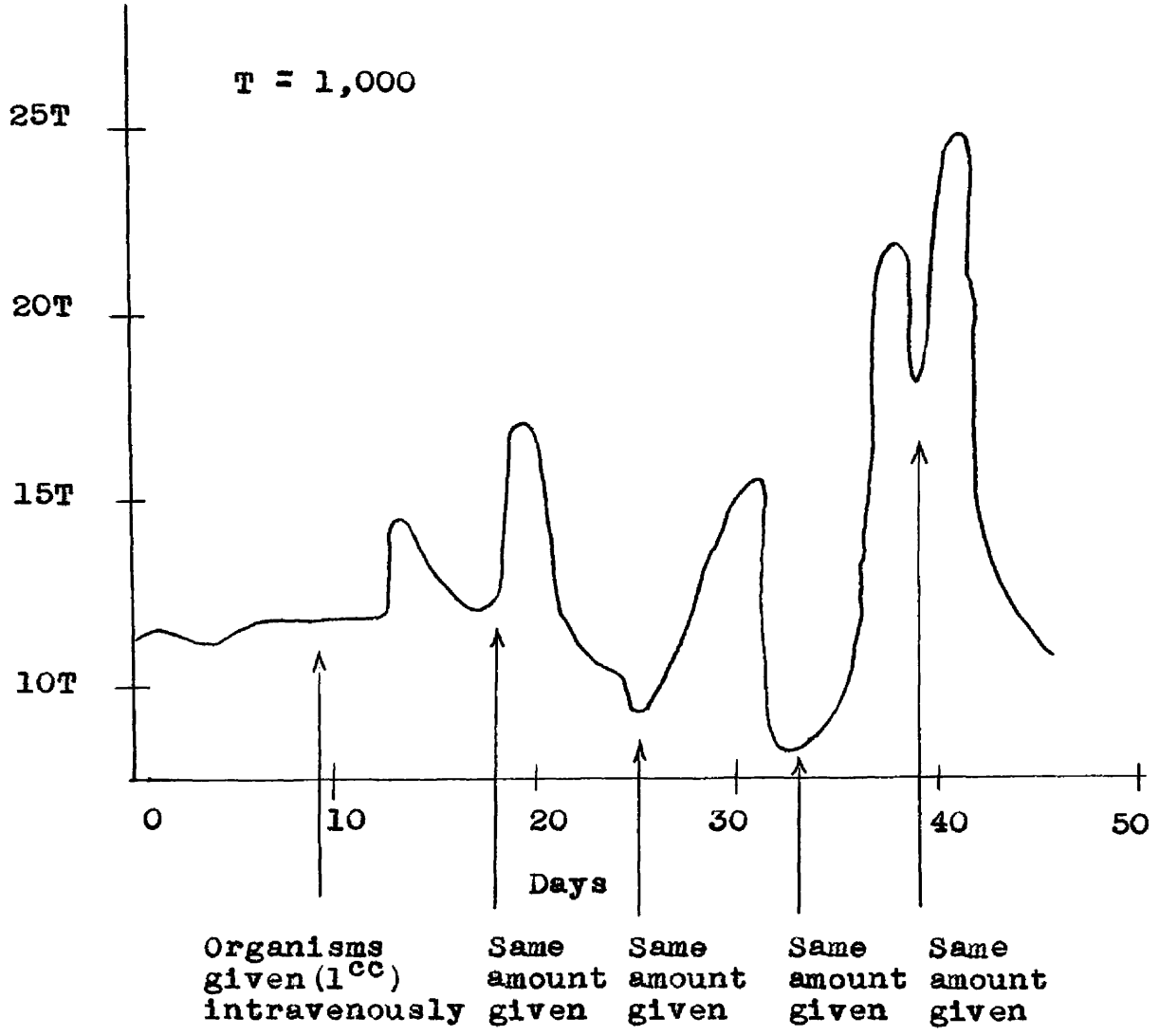


Figure 15

Number of red blood cells per cubic mm in turkey

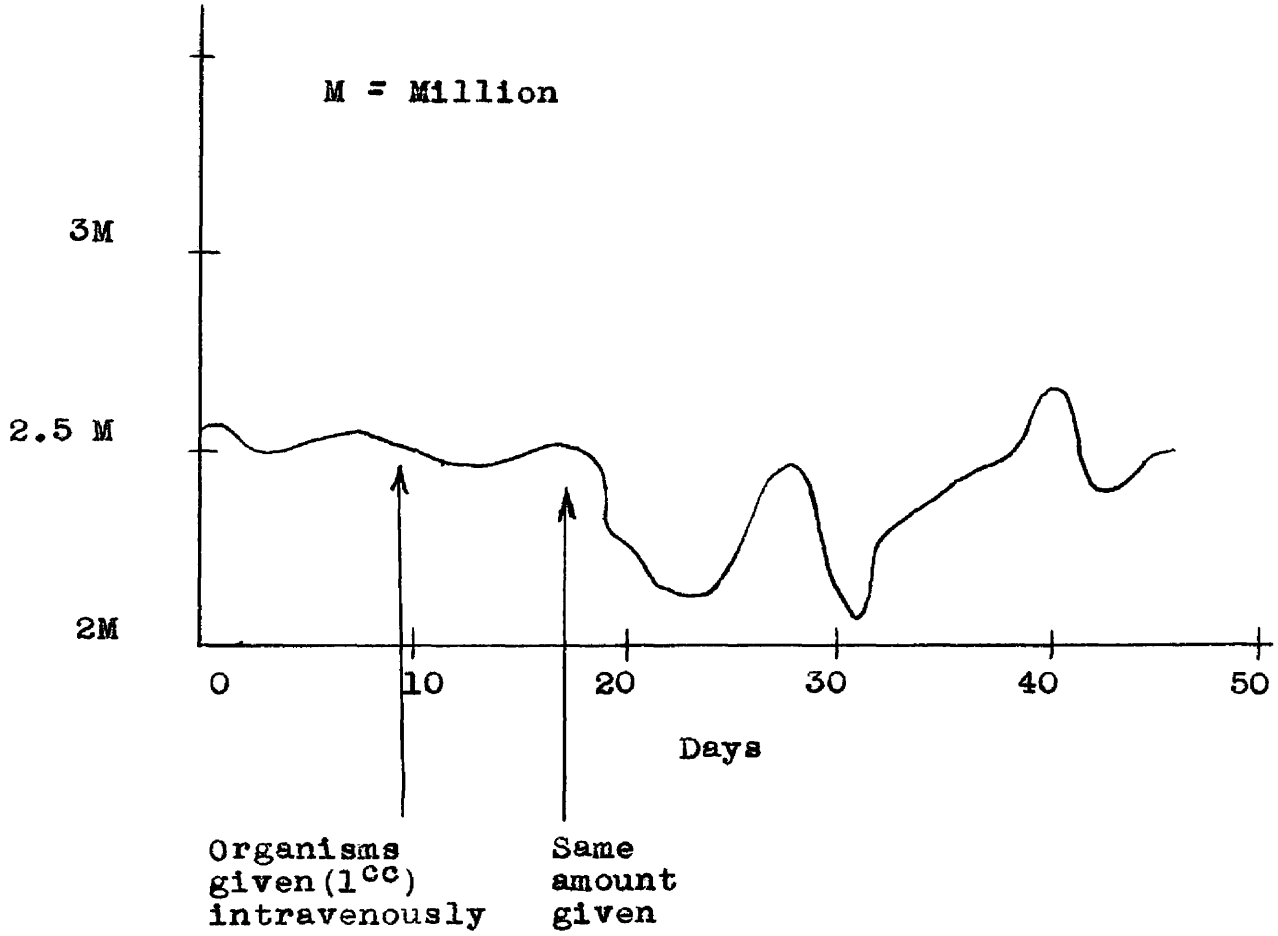
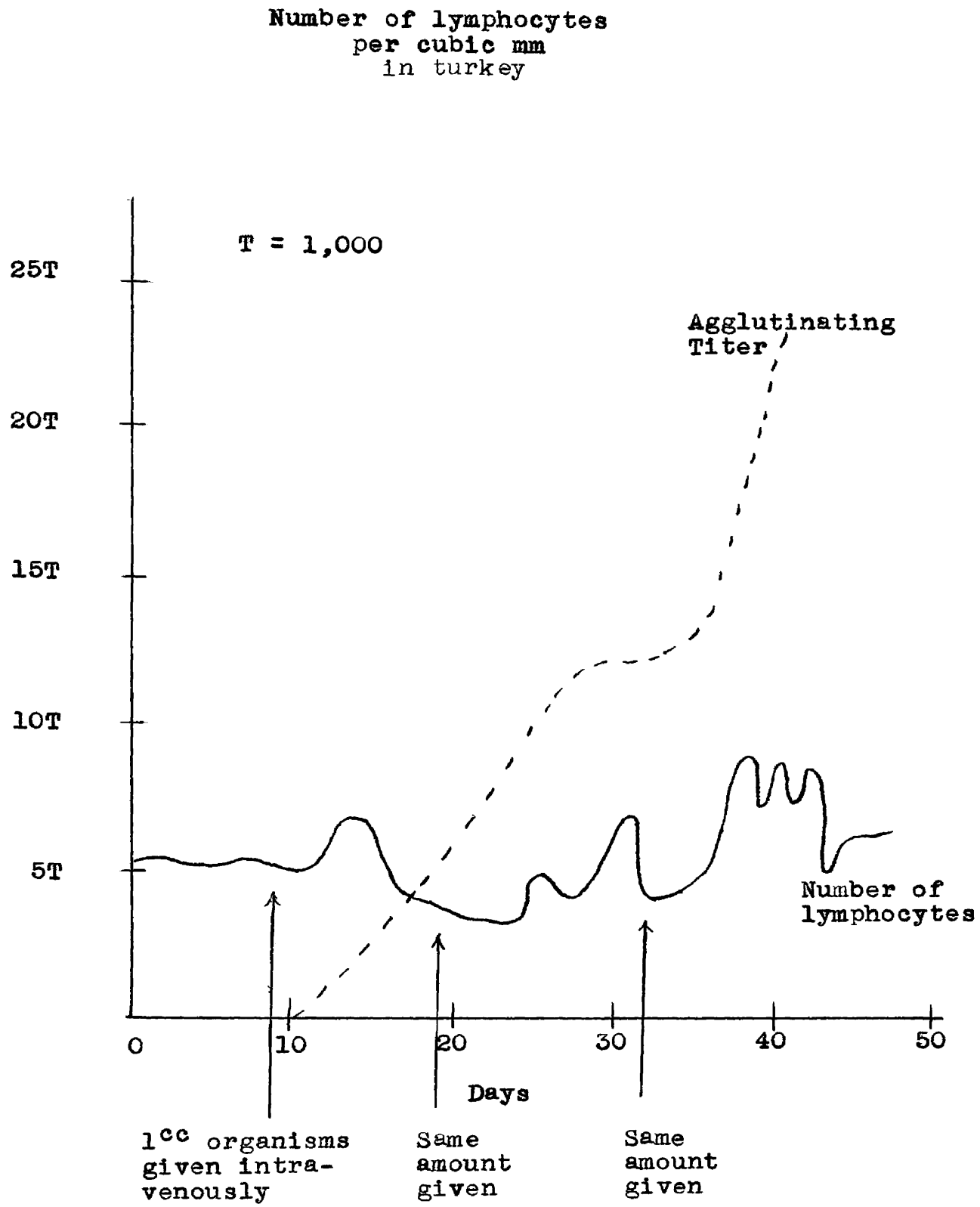


Figure 16


Number of lymphocytes per cubic mm in turkey
T = 1,000
25T
20T
15T
10T
5T
Agglutinating Titer
Number of lymphocytes
0
10
20
30
40
50
Days
1cc organisms given intra-venously
Same amount given
Same amount given