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INFECTION AND RESISTANCE IN THE BLOOD-INHABITING PROTOZOA[1]

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CERTAIN of the blood protozoa, because of their size and easily accessible location, offer unique opportunities for the study of the effects of acquired resistance on the parasites and of the humoral and cellular bases for these effects. This evening I propose to discuss two of these in detail, *Trypanosoma lewisi* of the rat and *Plasmodium cathemerium* of the bird, and to consider a few others comparatively. Specifically, I plan to describe the normal course of infection, to analyze the effects of the host's resistance on the parasites, to describe the antibody bases for some of these effects and to correlate the antibody and other immune responses with the cellular reactions of the host. I sincerely hope that these facts will be of intrinsic interest to you and in addition that they will illustrate the methodology which we have found successful in our immunological studies of protozoan infections. But, above all, I hope that the facts will bring out the peculiar advantages of these protozoa as material for certain immunological problems.

The fundamental methods which we have used in analyzing the effects of resistance will become apparent in the discussion of the specific infections. A few words should be said, however, regarding the method of reproduction in the blood protozoa and the meaning of changes in numbers of the protozoa throughout the course of an infection. Among the trypanosomes reproduction is practically limited to binary

[1] Harvey Lecture delivered before the New York Academy of Medicine, December 17, 1931.

fission. In malaria it involves schizogony, which is essentially a series of binary fissions of the nucleus followed by a terminal splitting of the cytoplasm. The organisms in both of these infections are limited to and are distributed throughout the blood stream. Therefore, if no factor or factors influence either the rate of their reproduction or their survival after they are produced, they should increase in the blood uniformly according to a geometrical progression. Conversely, the daily changes in the number of organisms per cmm of blood roughly measure the sum total of the resistance developed by the host, but they do not indicate how much of the resistance is due to an inhibition of reproduction of the parasites and how much is due to a parasiticidal effect which kills the parasites after they are produced. This evening I wish to stress the fact that this inherent difficulty can be overcome by devising various methods of ascertaining the basic rate of reproduction by measures which are independent of the number of organisms killed and the further fact that different immunological mechanisms are involved in the inhibition of reproduction and in the parasiticidal effect.

TRYPANOSOMA LEWISI

It is hardly necessary to recall to your mind that *Trypanosoma lewisi* is a non-pathogenic blood parasite of rats all over the world and is transmitted from rat to rat by various species of fleas. It is a comparatively large trypanosome (about 30 μ in length) and occurs in the peripheral blood stream without tissue localization. Thus, what is happening in an infection can be ascertained by a study of samples of the peripheral blood.

Daily number counts of the trypanosomes throughout the course of an infection in the rat show that the infection progresses with certain typical features. (See review in Taliaferro, 1929.[2]) Following the injection of the parasites, unless large numbers have been injected intravenously, there is an incubation period of greater or less time during which no organisms are found in the blood. Then there is an acute rise of the infection until the trypanosomes may reach 300,000 or more per cmm. This peak generally occurs between the 8th and 14th day whereupon the organisms markedly diminish in what I have termed a number crisis. Following this disappearance of most of the parasites the infection enters a developed phase during which there may be a gradual decrease in numbers, but no marked crisis. The developed infection may last from a few days to many months. Sooner or later, however, it is terminated by the more or less abrupt disappearance of the parasites from the peripheral blood stream (Fig. 1). The fact that there is no tissue localization allows certain conclusions to be drawn from these number counts. Undoubtedly there must be a trypanocidal factor killing the trypanosomes at the time of the first number crisis and at the end of the infection to account for the decrease in numbers, since even if the rate of reproduction were reduced to zero, it could not explain an actual decrease of the trypanosomes. The question then arises as to whether in addition to this trypanocidal agent there is also a factor influencing the rate of reproduction.

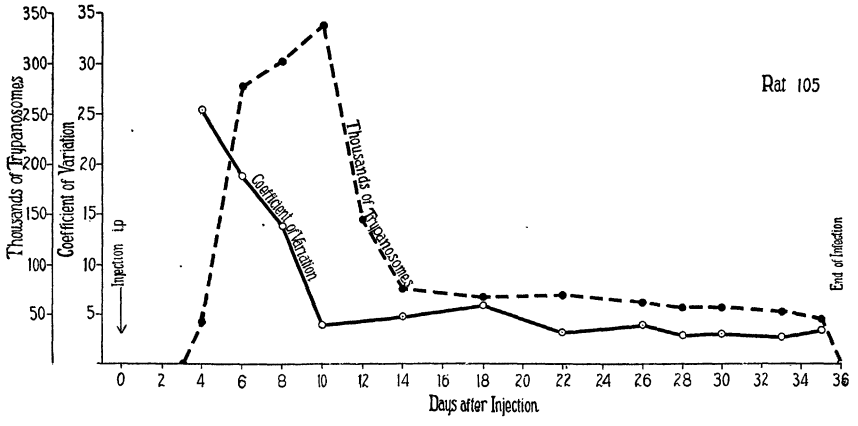

FIG. 1. Graph showing the three factors of resistance developed in a rat against an infection of *T. lewisi*. The two trypanocidal effects are represented by the two crises in the number curve and the reproduction-inhibiting effect is represented by the gradual drop in the coefficient of variation for total length of the trypanosomes. (From the author.)

A study of the parasites during the ordinary course of an infection indicates that the trypanosomes actually reproduce during only the first part of the infection. Thus, when the trypanosomes are intro-

[2] W. H. Taliaferro, "The Immunology of Parasitic Infections," New York, pp. 414, 1929.

duced into the rat they begin reproduction by active cell-division within about 48 hours, continue this reproduction at a maximum rate for several days, then at a decreasing rate for several days until approximately the 10th to 12th day of the infection they have ceased to reproduce and live in the blood stream for from a few weeks to many months simply as non-reproducing adults.

As stated in my introductory remarks this inhibition of reproduction is not demonstrated by number counts, but by methods not affected by trypanocidal factors. Two such methods have been devised. The simplest method consists of ascertaining the percentage of dividing forms found in daily blood smears. Such a procedure (Fig. 2) discloses that actual dividing trypanosomes occur until about the 8th day, that variable growth forms continue until about the 11th day and that only adult forms which are extremely uniform in size and structure and which never show any stages in the division of their organelles are found after the 11th or 12th day. This method of demonstrating the cessation of reproduction in the trypanosomes was known to the early microscopists and the results clearly depicted as early as 1899 by Rabinowitsch and Kempner,[3] by v. Wasielewski and Senn (1900),[4] and especially by Laveran and Mesnil (1901).[5] A somewhat more exact method was devised by Mrs. Taliaferro and me in 1922[6] which consists in ascertaining the coefficient of variation for the total length of the trypanosomes throughout the infection. The rationale of this method is based on the obvious fact that reproducing forms with the consequent production of small forms and growth stages are much more variable than non-reproducing adults. Thus, as can be seen in Fig. 1, when the trypanosomes were at the height of their reproductive activity, as demonstrated by microscopical examination, the coefficient of variation for their total lengths was approximately 25 per cent., whereas as they ceased to reproduce, the coefficient of variation proportionately decreased until when the parasites were non-producing adults, it shaded off to about three per cent.

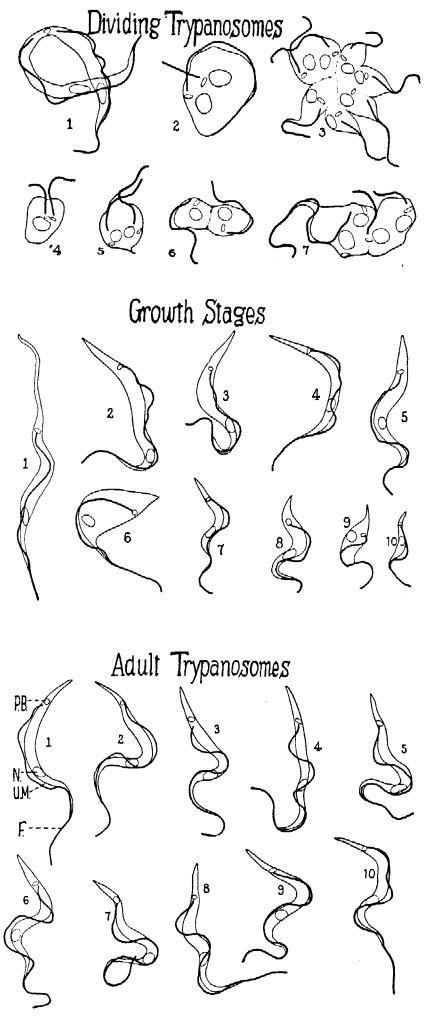

FIG. 2. Dividing, growth, and adult *T. lewisi*. The dividing and growth forms are rarely found after the tenth day of a normal infection. F., flagellum; N., nucleus; P. B., parabasal body; U. M., undulating membrane. ×1000 (Dividing forms, from Coventry; others, from the author.)

To sum up the effects of resistance on the trypanosomes, we may conclude that there is, first, some factor which completely inhibits all reproduction of the parasites by about the 10th day; second, some trypanocidal factor which kills the majority of the organisms at the time of the first number crisis (8th to 14th day); and third, a similar trypanocidal factor which terminates the infection at the end of a few days to several months.

The next step in analyzing the host's resistance to *T. lewisi* consists in demonstrating that the three effects, just outlined, are connected with definite humoral antibodies. The immunological basis for the inhibition of reproduction of the parasites was first demonstrated by the speaker in 1924[7] and was later confirmed by Coventry (1925)[8] and Regendanz and

[3] L. Rabinowitsch and W. Kempner, *Ztschr. f. Hyg. u. Infektionskr.*, 30: 251–294, 1899.

[4] v. Wasielewski and G. Senn, *Ztschr. f. Hyg. u. Infektionskr.*, 33: 444–472, 1900.

[5] A. Laveran and F. Mesnil, *Ann. de l'Ist. Pasteur*, 15: 673–714, 1901.

[6] W. H. Taliaferro and Lucy G. Taliaferro, *Amer. Jour. Hyg.*, 2: 264–319, 1922.

[7] W. H. Taliaferro, *Jour. Exper. Med.*, 39: 171–190, 1924.

[8] Frances A. Coventry, *Amer. Jour. Hyg.*, 5: 127–144, 1925.

Kikuth (1927).[9] Briefly stated, the serum of an infected rat, in which trypanosomes have ceased to reproduce, contains a passively transferable property which will prevent adult trypanosomes from reproducing in normal rats, but which apparently does not kill them or affect their vitality. One experiment will make this clear. On the 10th day after infection (when the trypanosomes had reached the adult stage), a rat was killed and bled and its serum containing adult trypanosomes collected. Next, the trypanosomes were separated from the serum by rapid centrifugation. Then half of the adult trypanosomes together with 2 cc of the serum (per 100 gm rat) were injected into an experimental rat, while the other half of the trypanosomes together with a similar dose of normal rat serum were injected into a control rat. Daily examinations of blood smears and calculations for the coefficient of variation for total length for each of these rats showed that the trypanosomes in the experimental rat lived in the blood for 11 days (when the infection ended) without showing any reproduction whatever, whereas in the control rat reproduction began on the second day and followed the normal course. Moreover, in the experimental rat, the trypanosomes did not increase in numbers, whereas in the control rat, they increased, etc., as usual. In this experiment it is to be noted that not only did the immune rat serum (*i.e.,* the serum taken from the seed rat on the 10th day of its infection) prevent adult trypanosomes from reproducing in a test rat, but normal rat serum failed to prevent adult trypanosomes from reproducing in a control rat. Here, we have a clear-cut passive transfer of this type of immunity from an infected to an uninfected rat.

Similarly, the first number crisis is associated with a trypanocidal antibody as was first demonstrated by Coventry (1930).[10] According to her work, serum taken after the first sudden drop in numbers of parasites, was curative (*i. e.,* caused the trypanosomes to disappear) when injected into rats in which the trypanosomes had just appeared in the blood. Such serum, however, was without effect if injected after the natural number crisis. It appears, therefore, that the parasites that survive the first number crisis are either basically non-susceptible or acquire a resistance to the antibody. Since many observers have shown that whenever a trypanolysin operates on the pathogenic trypanosomes, some of them always survive, become resistant and produce a relapse, it seems not improbable that the non-pathogenic *T. Lewisi,* surviving the action of the trypanocidal antibody at the crisis, becomes similarly resistant.

[9] P. Regendanz and W. Kikuth, *Centrabl. f. Bakt.,* Orig., 103: 271–279, 1927.

[10] Frances A. Coventry, *Amer. Jour. Hyg.,* 12: 366–380, 1930.

There has been some difference of opinion as to the mechanism whereby the rat eliminates the trypanosomes at the end of the infection. During recent years Regendanz and Kikuth (1927)[9] concluded that it was the result of a non-specific phagocytosis by the reticulo-endothelial system. On the other hand, a large mass of evidence indicates that it is due in major part to a specific antibody. Thus, as early as 1899, Rabinowitsch and Kempner[3] showed that the serum of recovered rats was highly protective (*i.e.,* prevented infection), a fact which was verified and greatly extended by Laveran and Mesnil (1901).[5] The latter also were able to establish that such serum sometimes, but not invariably, was curative as well. More recently Coventry (1930)[10] demonstrated that it was invariably curative provided the stage of infection at which the serum was administered was standardized. In a reinvestigation of the whole question during the last few years I have come to the conclusion that the final disappearance of the trypanosomes is associated with a highly active trypanocidal antibody which acts by killing the organisms and kills so quickly that the anti-reproduction factor does not even have time to act and which can be demonstrated either protectively or curatively. Some of the infections in some of the rats illustrate another fact which I have emphasized before, *viz.,* that the examination of a number curve alone does not necessarily give a conclusive answer as to whether reproduction is inhibited. Thus, in one animal, the infection was held down for 8 days and yet examination of the parasites showed them to be reproducing at a normal rate. From this, we can conclude that no anti-reproductive mechanism was operative, but that some trypanocidal mechanism was active either directly as a result of the administration of the immune serum or indirectly through the stimulation of the body's defense.

Although there is little question that the trypanocidal antibody occurs, there is still lack of agreement as to whether it kills by opsonization or by lysis. Thus, Laveran and Mesnil (1901)[5] considered that the parasites were actively phagocytosed, whereas MacNeal (1904),[11] Manteufel (1909)[12] and the author (1924)[7] considered that they were lysed. Since the contention of Wells (1929)[13] and others that an opsonin and a lysin are the same seems to me well grounded, I do not believe this specific question need worry us further.

I have described in *T. Lewisi* two markedly different effects of resistance, namely, the inhibition of repro-

[11] W. J. MacNeal, *Jour. Infect. Dis.,* 1: 517–543, 1904.

[12] Manteufel. *Arb. a. d. k. Gesundh.,* 33: 46–83, 1909.

[13] H. G. Wells, "The Chemical Aspects of Immunity." 2nd. New York, pp. 286, 1929.

duction and their destruction and have ascribed their action to antibodies. The question arises: Are these antibodies identical? After some seven years' study, I (in press)[14] now feel that they are essentially different. Both antibodies show the following similar characteristics. Both are associated with the activity of the spleen and other locations rich in reticulo-endothelial cells: both are precipitated with the globulin fractions of the serum: both appear in suitable animals after immunization with killed *T. lewisi:* both are specific for *T. lewisi;* and both arise as a result of infection of an animal with *T. lewisi.* They exhibit the following differences. The reproduction-inhibiting antibody inhibits cell-division, but does not kill the parasites or induce them to become resistant, and it shows no persistent union with the trypanosomes *in vitro* so that neither is the property absorbed by the trypanosomes nor are the tryponasomes sensitized against reproduction as tested by washing them free of the serum and injecting them into normal rats.

The lack of *in vitro* affinity between the reproduction-inhibiting antibody and the specific antigen, *i. e., T. lewisi,* is particularly interesting and should be emphasized. It is comparatively easy to show the *in vitro* affinity of *T. lewisi* for its specific trypanocidal antibody. Thus, in a typical experiment, serum, which was obtained after the natural termination of the infection, when tested *in vivo* was trypanocidal in curative experiments in doses of 1.0 cc to 4.0 cc per 100 grams rat and in protective experiments in doses of 2.0 cc to 5.0 cc. After absorption with dividing and adult trypanosomes—about 1,000,000 trypanosomes per cmm—there was no trypanocidal action in curative experiments in doses as high as 4.0 cc nor in protective experiments with one exception, in doses as high as 6.0 cc. The trypanosomes used for this absorption were highly sensitized so that they lived only a few minutes when introduced intravenously into a normal rat. In marked contrast to this, reproduction-inhibiting serum (*i. e.,* serum taken after the inhibition of reproduction, but before the termination of the infection) when tested *in vivo* elicited inhibition of reproduction of trypanosomes in doses of 1.5 cc to 4.0 cc per 100 grams rat, whereas after absorption with dividing trypanosomes its titer was exactly the same. Similar results were obtained when the serum was absorbed with adult trypanosomes and with both adult and dividing trypanosomes. Furthermore, the trypanosomes used in attempting to absorb the serum were not sensitized against reproduction because when injected into normal rats they underwent their usual cycle of reproduction. The objection might be raised that sufficient trypanosomes had not been used. Two facts militate against such an objection. In the first place, if too few were used those that were used would have been very highly sensitized by the antibody. But they were not. In the second place, I have found it possible to take serum after the termination of the infection which contains both the trypanocidal and the reproduction-inhibiting antibody and by absorption to remove the one and leave the other. In the light of this work and in the interest of a more concise terminology I[14] have just recently designated the principle in serum which inhibits cell-division of organisms but does not kill them as *ablastin,* from the Greek *blastos,* a sprout, germ, offspring and *ablastos,* not budding, barren, etc. Parenthetically I may add that I have always favored the unitarian hypothesis that the various serological manifestations exclusive of antitoxin-toxin reactions, are due to a single antibody. Although the trypanocidal antibody is undoubtedly a manifestation of this single postulated antibody, I believe, that the work just reviewed indicates that the reproduction-inhibiting antibody is essentially different.

As has been previously noted, the reproduction-inhibiting antibody or *ablastin,* is associated with the activity of the spleen and other locations rich in reticulo-endothelial cells. This fact is of inherent interest and has elicited considerable work. In 1927, Regendanz and Kikuth[9] showed that removal of the spleen of trypanosome-infected rats often caused a lengthening of the reproduction of the trypanosomes which they interpreted as following from a decrease in the formation of this specific antibody. Marmorston-Gottesman, Perla and Vorzimer,[15, 16, 17] in a series of papers published in 1930, corroborated and extended these findings. These results were somewhat complicated, however, by the fact that both groups of investigators used rats which were infected with *Bartonella muris-ratti,* although the first investigators tried to control the infection with neo-salvarsan. Infection with this organism will remain latent and unnoticed ordinarily, but will flare up with intense anemia upon any pronounced disturbance of the reticulo-endothelial system, such as splenectomy. In other words, infections with both *Bartonella* and *T. lewisi* are intimately connected with the action of the reticulo-endothelial system. Consequently, in collaboration with Cannon and Goodloe, I (1931)[18] re-examined the relationship of the spleen and the gen-

[14] W. H. Taliaferro, *Amer. Jour. Hyg.* (in press), 1932.

[15] J. Marmorston-Gottesman, D. Perla and J. Vorzimer, *Jour. Exp. Med.,* 52: 587–600, 1930.

[16] J. Marmorston-Gottesman and D. Perla, *Jour. Exp. Med.,* 52: 121–129, 1930.

[17] D. Perla and J. Marmorston-Gottesman, *Jour. Exp. Med.,* 52: 601–616, 1930.

[18] W. H. Taliaferro, P. R. Cannon and Sara Goodloe, *Amer. Jour. Hyg.,* 14: 1–37, 1931.

eral macrophage system to the formation of the reproduction-inhibiting antibody in *Bartonella*-free as well as *Bartonella*-infected rats and found that it was demonstrated by histological changes, by splenomegaly and by various experimental procedures known to affect the reticulo-endothelial system. Thus, rats infected with *T. lewisi,* but *Bartonella*-free, showed in the spleen an increased prominence of the follicles and marginal zones, and a mild hyperplasia of both red and white pulp cells. These cellular changes were not as pronounced as those found when *Bartonella* alone was present (Cannon and McClelland, 1929)[19] and were even less pronounced than when *Bartonella* was present in conjunction with *T. lewisi.* Moreover, this work demonstrated that when such things as splenectomy, *Bartonella*-infection, paratyphoid infection, India ink blockade and pregnancy occur separately in a rat infected with *T. lewisi,* the reproductive cycle of the trypanosomes is not materially influenced, but that when two or more occur in conjunction with *T. lewisi* in the same rat, the reproductive activity of the trypanosomes is often profoundly altered. To illustrate—in one rat which was infected with *Bartonella* the trypanosomes underwent their usual cessation of reproduction in the initial infection, but when splenectomy was performed, an intense relapse of reproduction became evident within three days after the operation and during the later stages of pregnancy, a similar relapse occurred.

Taken as a whole, this work indicates that the macrophage system in rats infected with *T. lewisi* but otherwise healthy has an effective functional level so that by the 8th to the 12th day of the infection all reproduction of the trypanosomes is inhibited and continues to be inhibited throughout the remainder of the infection, that this functional level is lowered in a cumulative way by splenectomy, *Bartonella*-infection, paratyphoid infection, India ink blockade and pregnancy, so that any one alone produces no significant derangement, but two or more in combination elicit profound disturbances in a high percentage of rats.

A similar relationship between the reticulo-endothelial system and the trypanocidal antibody in *T. lewisi* can be demonstrated, but I have stressed the relationship between the reticulo-endothelial system and the reproduction-inhibiting antibody because it is so clear-cut and gives such a unique tool for the study of the reticulo-endothelial system, not only in connection with its relation to immune phenomena, but also in establishing its connection with various general metabolic processes. Thus, we hope to infect rats with trypanosomes, wait until their reproduction has ceased and then subject them to various physiological disturbances, such as dietary insufficiencies, hormone administration, fatigue and numerous other factors to see if the trypanosomes reinitiate reproduction. If they do, we feel that we are justified in concluding a direct interrelationship of such physiological factors and the reticulo-endothelial system.

I shall not consider other species of trypanosomes in detail, but certain comparative facts should be brought out. In no case so far studied is the reproduction-inhibiting antibody or ablastin formed in infections with the pathogenic trypanosomes.[2,6] In fact, that appears to be the outstanding difference between the two groups. Thus, when *T. brucei, T. gambiense, T. rhodesiense, T. equiperdum* and related forms are grown in mice there is no evidence of the action of either trypanocidal or reproduction-inhibiting factors. When the same forms are grown in the guinea-pig no reproduction-inhibiting factor is operative, but periodic trypanolysins are liberated in the blood and are associated with decreases of the parasites. Much the same probably holds for sleeping sickness in man. These trypanolysins are not permanently effective, however, because as a rule all of the trypanosomes are not killed and the survivors become resistant to the antibody. Being resistant and having their basic rate of reproduction unchanged, they repopulate the blood stream in one or more relapses until the animal succumbs.

Ablastin undoubtedly plays an important part in the prevention of relapses. Thus, during the first part of the infection with *T. lewisi,* the reproduction of the parasites is first reduced to zero. Therefore, when a trypanocidal factor appears it permanently reduces the parasites in the blood because those that remain can not reproduce, and hence can not reaccumulate or produce a relapse, as they do in the pathogenic infections. Similar antibodies occur in a group of non-pathogenic trypanosomes closely related to *T. lewisi,* but have not been found in a number of other infections which we have studied.

Plasmodium cathemerium

As a second infection to consider in detail, I have selected the frequently non-lethal malarial parasite, *Plasmodium cathemerium,* of the sparrow which can be experimentally studied in the canary. In this infection, unlike infection with *T. lewisi,* only a parasiticidal type of resistance is developed and furthermore, no intermediate action through a humoral antibody has been demonstrated, although the parasiticidal mechanism is again associated with the macrophage system in what Gay (1931)[20] has aptly referred to as a "pure histologic immunity."

The peculiar synchronous method of reproduction

[19] P. R. Cannon and P. H. McClelland, *Arch. Path. and Lab. Med.,* 7: 787–800, 1929.

[20] F. P. Gay, *Jour. Amer. Med. Ass.,* 97: 1193–1199, 1931.

found in the malarial parasites allows us to carry our analysis of the factors in resistance somewhat further than in the case of *T. lewisi.* I need only recall to your mind that, whereas reproduction in the trypanosomes is haphazard, in the malarial organisms it is synchronous. Thus, all the young asexual parasites (which are the typically vertebrate part of the life-history of this form) grow up and sporulate more or less simultaneously so that at any one time a preponderance of one particular stage will be found in the blood. In the case of *Plasmodium cathemerium,* according to L. G. Taliaferro (1925)[21] and others, this asexual cycle takes 24 hours. As shown in Fig. 3,

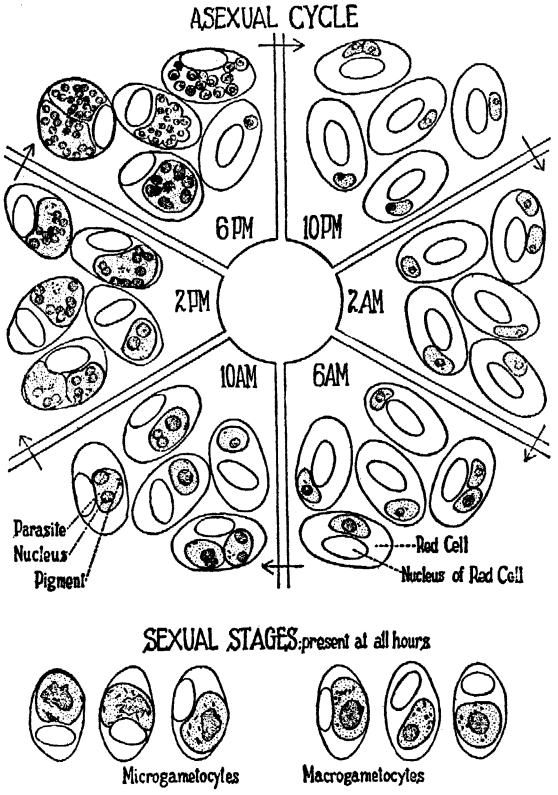

FIG. 3. Outlines of the asexual stages of *P. cathemerium* showing the synchronous method of reproduction occurring every 24 hours. ×1350. (From L. G. Taliaferro.)

the young parasites start their existence about 6 P. M., grow, undergo a series of nuclear divisions and 24 hours later break up, *i. e.,* sporulate, into about 15.5 small parasites. Thus, the mean size of random samples taken throughout the day will show this cycle inasmuch as the mean size is small when there are only merozoites and increases until it reaches a peak just before the mature schizont breaks up during sporulation. From the standpoint of our present study the length of this asexual cycle, which is the time it takes one parasite to become 15, is a direct measure of the rate of reproduction of the parasites since the parasites always segment into approximately the same number, and it is a measure which is independent of parasiticidal factors.

Superficially the course of the infection of *P. cathemerium* in the canary is very similar to *T. lewisi* in the rat. Thus, there is an early acute rise in the number of parasites which reaches its peak between the 8th to the 15th day and which is followed in turn by a sharp number crisis, by a period of developed infection when there are comparatively few parasites in the blood, and by a latent stage which may last for as long as four years and during which there are so few organisms in the blood that they can rarely be found by microscopic examination, but can be demonstrated by injecting large amounts of blood into uninfected birds. Throughout this latent period severe relapses may occur (See the Sergents, 1918,[22] Ben Harel, 1923,[23] L. G. Taliaferro, 1925,[21] Boyd, 1924[24] and Hartman, 1927.[25]) Just as in *T. lewis,* there are no tissue localizations so that from the number curve alone we are justified in concluding that some parasiticidal mechanism eliminates most of the parasites at the time of the crisis.

When the rate of reproduction of the organisms is studied, however, we find an entirely different picture from that in *T. lewisi.* L. G. Taliaferro (1925)[21] found that the rate of reproduction of the parasites remained constant during the acute, chronic and relapse periods. In other words, the parasites reproduced every 24 hours whenever they could be found

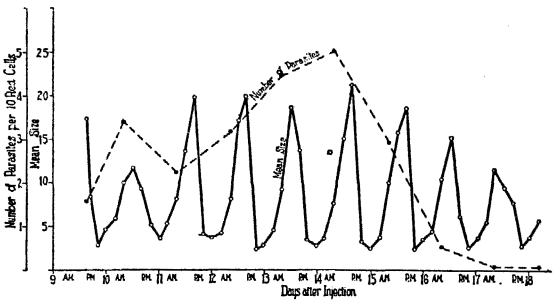

FIG. 4. Graph showing the plasmodicidal factor of resistance developed in a bird against an infection with *P. cathemerium* as represented by the crisis in the number curve. There is no reproduction-inhibiting effect as shown by the regularity of the mean-size curve, *i.e.,* every 24 hours the parasites sporulated. (From L. G. Taliaferro.)

[21] Lucy G. Taliaferro, *Amer. Jour. Hyg.,* 5: 742–789, 1925.

[22] Ed. Sergent and Et. Sergent, *Ann. de l'Inst. Pasteur,* 32: 382–388, 1918.

[23] S. Ben Harel, *Amer. Jour. Hyg.,* 3: 652–685, 1923.

[24] G. H. Boyd, *Amer. Jour. Hyg.,* 5: 818–838, 1925.

[25] E. Hartman, *Amer. Jour. Hyg.,* 7: 407–432, 1927.

in the blood for study. Fig. 4 shows the regularity of the mean curve during the major portion of the acute period, the crisis and the beginning of the latent period of an infection. From this it follows that there is no reproduction-inhibiting effect of resistance such as exists in infections with *T. lewisi*.

The parasiticidal effect may now be considered in detail. As was previously indicated, the average number of merozoites formed from one parasite was found to be 15.5 and on *a priori* grounds the daily rate of increase of the parasites during the acute period should be the same. Number counts, however, revealed that the increase was only by 5. In other words, each schizont produced a brood of approximately 15 parasites, but ten of them died before completing their reproductive cycle. This rate of death occurred immediately after the bird was infected and was constant throughout the acute period. It does not, therefore, represent an acquired resistance, but probably is a measure of the suitability of birds for the malaria parasite and represents in a sense a natural immunity. Whether or not it is the result of active processes in the bird, such as specific phagocytosis, or is a natural death rate of the parasites, as suggested by Hartman (1927),[25] has not been definitely proven.

At the time of the crisis instead of 10 parasites out of each brood of 15.5 being killed, the rate of death of the parasites was far in excess of the net rate of reproduction. This represents an acquired resistance resulting from infection which is effective to a greater or less extent in holding the parasites down throughout the remainder of the infection.

That it is preeminently efficient has been demonstrated and directly measured by injecting parasites into a latent bird and comparing their fate with similar parasites injected into a normal bird. One experiment by the speaker and L. G. Taliaferro (1929 a)[26] will illustrate the results obtained. An experimental bird, that is, an immune bird, on the forty-third day of its primary infection when there were no longer parasites in its blood, and a normal bird were given similar intravenous doses of parasites at 1:00 P. M. so that a few minutes later there were 8 parasites per 10,000 red blood cells in their blood. The sequence of events in the two birds was strikingly different. Whereas in the experimental bird the parasites had disappeared by 10:00 A. M. the day after the intravenous injection and never reappeared (the bird died on the eighth day of the experiment), in the control bird they showed a typical course of infection. The results were even more dissimilar when larger doses of parasites were given, for the immune birds disposed of their parasites so that they could no longer be found 2 to 3 days after injection, whereas the normal birds died between the fifth to eighth day from the ensuing overwhelming infection with approximately every other cell parasitized.

Other conclusions from the same paper may be briefly summarized as follows: There is a high degree of immunity to superinfection which begins as soon as the latent period sets in and lasts for extremely long periods. Thus, birds with latent infections that had been inoculated from 16 to as long as 656 days previously, when superinfected, disposed of the parasites quickly, whereas normal birds could not cope with such enormous numbers and quite frequently died. The removal of the parasites in the latent birds depends to a certain degree on the number injected. In other words, when approximately 1 to 100 parasites per 10,000 red blood cells were introduced, they were removed from the peripheral blood within 24 hours, whereas when from 100 to 400 per 10,000 red cells were introduced, they were removed in from 48 to 76 hours. The parasite-red-cell complex is removed throughout the asexual cycle and not simply the merozoites free in the serum, as is evidenced by the fact that when hourly blood smears were made following the intravenous injections the decrease in numbers was gradual and not restricted to the time of sporulation. The ability to remove the parasites is very labile and very delicately attuned to the physiological condition of the bird and may be upset upon the slightest provocation. Thus, of six birds with latent infections, when injected intravenously 33 days after the initial inoculation with the same number of parasites, one had removed them from the blood by the following day, two got rid of them in two days, whereas the other three took three days to dispose of them.

We (W. H. and L. G. Taliaferro, 1929b)[27] then endeavored to find an antibody basis for this parasiticidal activity and naturally looked for some type of antibody similar to the ones easily demonstrated in the trypanosome infections. In this we completely failed. Thus, in six birds which were given varying doses of serum up to 1.05 cc, which is more than the amount obtainable from one bird, the infection showed up within from 1 to 6 days, whereas similar infections in normal birds showed up in from 1 to 9 days. This and other experiments led to the conclusion that immune serum, *i.e.*, serum recovered from birds during a latent malarial infection, was without curative, protective or opsonizing effect on the parasites. Nevertheless, at the close of this talk, I shall

[26] W. H. Taliaferro and Lucy G. Taliaferro, *Jour. Prev. Med.*, 3: 197–208, 1929a.

[27] W. H. Taliaferro and Lucy G. Taliaferro, *Jour. Prev. Med.*, 3: 209–223, 1929b.

present evidence which still makes me feel that either some type of antibody must be present or the macrophages must be specifically changed.

The absence of an antibody basis for the high grade immunity to superinfection and the subsequent natural recovery from avian malaria made it all the more interesting to study the cellular basis for the effects we have described. This phase of the work has been done in collaboration with Professor P. R. Cannon, of the department of pathology of the University of Chicago (1931).[28] A study was made first of normal birds and of the cellular changes occurring in infected birds throughout the course of infection and these changes in turn were correlated with the various periods of the infection. Second, a comparison was made between the cellular reactions immediately following the introduction of a large number of parasites into a normal bird and into a latent, *i.e.*, immune bird. This second study gave a picture of the cellular basis for the parasiticidal mechanism that we have been considering.

The cellular reactions throughout the course of normal infections presented a picture of increasing activation of the mesenchyme which reached its height at the time of the crisis and continued to a lesser degree throughout the entire latent period of the infection. This activation of the mesenchyme was not only indicated by the increased mitoses and increased numbers of the undifferentiated lymphoid cells, but by the heightened phagocytic activity and increased numbers of the differentiated macrophages, particularly of the spleen and liver. Furthermore, the spleen was greatly enlarged.

An occasional phagocytosed parasite was found in the spleen and liver as soon as four hours, and thereafter, phagocytosis was constant and continued throughout the acute period—thus, directly verifying the decrease of the asexual forms as indicated by number counts. Evidences of activation first appeared in about 18 hours, were quite pronounced by 24 hours and then were increasingly apparent until they reached a climax between the eighth and tenth days concomitantly with the crises of the infection. At the time of the crisis, when so many parasites were being swept from the general circulation, the Kupffer cells in the liver were swollen and contained large numbers of malarial organisms and there was a striking increase in mitosis and in the number of mononuclear cells. The liver cords were notably disoriented. Similarly in the spleen there was a larger proportion of pulp cells (macrophages) containing malarial organisms coupled with a diffuse hyperplasia of lymphoid cells and a great increase of basophilic lymphoid cells. After this high point of activation, there was a gradual decline in phagocytosis and activation. This probably follows from the fact that once the body has gotten the infection in hand, there are comparatively few parasites present for removal. Nevertheless, that the altered reactivity is maintained is demonstrated conclusively when a large number of parasites is injected. Under such conditions phagocytosis is much more rapid and effective than in birds infected initially. In fact, it is well initiated within fifteen minutes after superinfection and within from 24 to 48 hours has so successfully operated that parasites can no longer be found in the peripheral blood. It is interesting to note that throughout our study the actual phagocytosis of the parasitized red cells is limited to the macrophages, that is, to the cells known under such various names as the hemophages of Kyes, the Kupffer cells in the liver, the pulp cells in the spleen, etc.

With this background it is possible to state very briefly our results on the cellular basis for immunity to superinfection. It will be recalled that when parasitized red cells were introduced into normal birds no appreciable phagocytosis of the organisms occurred before four hours and no distinct activation of the mesenchyme before 18 hours. In marked contrast to this when similar parasitized red cells were introduced into immune birds, phagocytosis was well initiated within 15 minutes and practically all of the parasites were removed within from 24 to 48 hours. The parasiticidal mechanism, then, involved in the destruction of the parasites at the time of the crisis, during the post-critical period, during latency and during immunity to superinfection, is primarily a cellular response of the host involving an activation of the mesenchyme. This activation, furthermore, is effective through two factors: (a) an actual increase in the number of phagocytic cells and (b) a greatly increased rate of phagocytosis by the individual macrophages.

The picture might be considered more or less complete were the activation of the mesenchyme non-specific, since then, of course, the absence of opsonizing antibodies would be accounted for, but Gingrich (1930)[29] has demonstrated that between *P. cathemerium* and *P. elongatum* the immunity is specific. Thus, during a latent infection with *P. cathemerium* there was an immunity to a superinfection of *P. cathemerium,* but not to an initial infection of *P. elongatum,* and *vice versa.* This demonstrates that the bird during latency acquires some mechanism which causes its phagocytes to ingest specific parasites and suggests either the production of a tropin

[28] P. R. Cannon and W. H. Taliaferro, *Jour. Prev. Med.*, 5: 37–64, 1931.

[29] W. Gingrich. Dissertation. Johns Hopkins School of Hygiene and Public Health, 1930.

which we have failed to demonstrate in our serological experiments, or a qualitative change in the macrophages. This whole question of the specificity of cellular responses in acquired immunity has been ably discussed in an earlier Harvey Lecture by Professor Gay (1931).[20]

The question immediately arises: Does the picture which I have presented for avian malaria hold for malaria in man and other mammals? At the present time Mrs. Taliaferro and I are carrying out a similar study on *P. brasilianum* of Panamanian monkeys which is a quartan parasite almost identical with *P. malariae* of man. Similarly, Professor P. R. Cannon is making a study of the cellular reactions of the monkey to this infection. It is too soon to give you the final results of this work, but the following preliminary conclusions are justified. The entire picture of the ordinary course of the infection is similar to that in the bird with certain time differences. There is no indication of any inhibition of reproduction of the parasites, but the number curves show that plasmodicidal factors are operating. So far we have not been able to correlate the killing of the parasites with any plasmodicidal antibody, but only with the direct phagocytosis of the parasites by the differentiated macrophages. The cellular responses during the initial infection are similar to those in birds. There are increasing evidences of activation of the mesenchyme during the acute rise of the infection which reaches its height at the time of the crisis and slowly subsides as the developed infection progresses and latency is initiated. There is also a high degree of immunity to superinfection during latency, which can be demonstrated by the injection of washed parasitized cells into monkeys with a latent infection, but so far no work has been done on the cellular basis for this immunity.

In comparing the immune reactions in infections with *T. lewisi* and *P. cathemerium* it is quite remarkable that the immediate mechanisms of immunity and effects on the parasites are so different, and yet, that in both cases the immunity can be eventually ascribed to the macrophage system. Thus, in one case the immunity is associated with an antibody which inhibits reproduction and with one or more trypanocidal antibodies, whereas in the other case there is no inhibition of reproduction and the parasiticidal mechanism consists of direct phagocytosis without the intervention of any humoral tropin that has been demonstrated up to the present.

These investigations on the cellular phases of immunity to protozoan infections are but one phase of the growing mass of evidence indicating the rôle of the macrophages in general and local immunity, in antibody production, in chemotherapy and in normal metabolism (see Linton, 1929;[30] Jungeblut, 1930,[31] and Gay 1931).[20] There are many obvious similarities between the work which I have presented and these subjects. I would like particularly to mention the following: The cellular responses to malaria show a striking similarity to the morphological changes described by Epstein (1929)[32] in the spleen and liver of the rabbit following the injection of such non-infectious antigens as sheep cells and horse serum. They parallel very closely the conditions found in other infections, such as *Bartonella,* to cite a single example in which the cellular responses have been very accurately studied (see particularly Cannon and McClelland, 1929;[19] Eliot and Ford, 1929,[33] and Marmorston-Gottesman and Perla, 1930[16]). And finally, although the cellular responses to malaria give rise to a general immunity, the same responses are probably elicited in so-called local immunity (see Gay, 1931[20]).

Conclusion

In concluding I wish to draw particular attention to the peculiar value of the blood protozoa for the study of what I believe to be some of the fundamental problems of immunity. All of these problems are related to the two great questions of how the host responds to infection and how the host's responses affect the invading organisms. The advantages of the protozoa in studying these questions may be briefly summarized as follows:

(1) *The analysis of the effects of resistance on the invading organism.* The fact that some protozoa live in and are evenly distributed through the blood stream allows the study of the course of infection from day to day. The further fact that the protozoa are comparatively large permits the study of cell-division and their general behavior throughout the course of infection. Specifically, I have shown that taken together such studies differentiate sharply between those factors in the host's resistance which kill the parasite, *i.e.,* parasiticidal factors, and those factors which inhibit cell-division, *i.e.,* reproduction-inhibiting factors. Although parasiticidal factors, such as trypanolysins and direct phagocytosis of organisms are well known, we have been able to demonstrate in one infection a peculiar type of antibody-response which does not kill the organisms, but which inhibits cell-division. These methods of analysis probably can not be applied to the smaller bacterial invaders although we have had some success in applying them to spirochaetes.

(2) *The action of antibodies on the protozoa.* The

[30] R. W. Linton, *Arch. Path.,* 6: 488–501, 1929.
[31] C. W. Jungeblut, *Ergeb. Hyg. Bakt. Immun. u. Exp. Therap.,* 11: 1–67, 1930.
[32] E. Epstein, *Virchows Arch.,* 273: 89–115, 1929.
[33] C. P. Eliot and W. W. Ford, *Amer. Jour. Hyg.,* 10: 635–642, 1929.

same characteristics of the blood protozoa listed above permit the direct study of the action of antibodies on the invading protozoa *in vivo*. Thus, when an immune serum is tried curatively, it can be ascertained by direct blood examination whether the organisms are killed or not. Similarly, the action of the reproduction-inhibiting antibody can be observed, that is, the presence or absence of cell-division can be directly ascertained. Here again the only methods available for similar studies with the smaller bacterial invaders are at best indirect.

(3) *The cellular basis of immunity.* The blood protozoa have proved admirable material for the correlation of cellular responses with immunity, antibody formation, etc. Thus, in the study of the production of the reproduction-inhibiting antibody in *T. lewisi* infections, it has been possible to use the trypanosomes as a delicate measure of the activity of the macrophage system. Similarly, in the acquired immunity of birds to malaria it has been possible to correlate the course of infection and immunity with the cellular responses. In fact, the malarial organisms have proven to be particularly advantageous for this type of study not only because they are large and can be easily found in the tissues, but also because even after digestion they leave a landmark for a considerable length of time in the form of the less readily digested pigment.

Lest I be misunderstood as suggesting the use of the blood protozoa for the study of all of the fundamental problems of immunology, I should also like to point out some of their shortcomings. Not even all the blood protozoa can be used for the studies I have presented this evening. Some of them, such as *T. cruzi,* the causative agent of Chagas's disease, form reproductive centers and localizations in the tissues which make the study of the normal course of infection by blood examinations impossible. Others, such as *P. falciparum,* the causative agent of estivo-autumnal malaria in man, although confined to the blood, show localizations in the capillaries of certain organs which thereby prevent the study of the course of normal infections by routine peripheral blood samples. The fact that the protozoa used in these studies are distributed throughout the blood stream prevents their use for studies in local immunity. Finally, inadequate cultural methods eliminate both the blood protozoa in particular and all protozoa in general as good material for the study of the serology and chemistry of immunological processes.

OBITUARY

ASHE, PIONEER FORESTER AND BOTANIST

THE death of William Willard Ashe on March 18, 1932, removes another distinguished name from the fast dwindling ranks of foresters who received their training in an era when the country was without established schools for education in the forestry profession. Ashe was born in Raleigh, N. C., on June 4, 1872, the oldest of nine children born to the Honorable Samuel A'Court Ashe and Hannah Emerson (Willard) Ashe. Ashe was noted as a boy for his love of nature, versatility, originality and for his mechanical and artistic ability. He was educated at the Raleigh Male Academy, the University of North Carolina (B. Litt., 1891), and Cornell University (M. S., 1892), specializing in geology and botany. He studied medicine for a while under Dr. W. I. Royster, of Raleigh. He was a member of the Sigma Alpha Epsilon fraternity. From 1892 to 1905 he served as forester of the North Carolina Geological Survey under Dr. J. A. Holmes. From 1905 until his death Ashe was an officer of the U. S. Forest Service, rising steadily in rank from forest expert and forest assistant to assistant district forester and senior forest inspector in "Region 7" (eastern United States). In 1906 he married a widow and distant cousin, Margaret Henry Wilcox. He had no children. Ashe was secretary of the National Forest Reservation Commission and editor of its reports from 1918 to 1924. He was elected vice-president of the Society of American Foresters in 1919. He became a member of the Forest Service tree name committee in 1928 and was chairman from 1930 to his death.

Ashe was a true pioneer. He was one of the real fathers of the forest acquisition policy of the federal government and was among the first to recognize the need for forest research in this country. He planted one of the first commercial stands of longleaf pine in North Carolina and discovered the secret of its successful transplantation. He is credited with introducing the modern cupping system in the American naval stores industry.

A bibliography of Ashe's scientific papers compiled by the writer covers 166 titles. He wrote extensively on systematic botany, logging costs, profitable forest management, land acquisition for conservation purposes, forest influences and forest types. One of the last papers he issued was a monograph of the genus Polycodium.

Ashe was an indefatigable observer, collector and annotator of plants. He published 510 new names in 35 genera. His taxonomic interests were (aside from his early papers in Asarum and Panicum) largely in connection with woody plants of the Southeast; 86 per cent. of his botanical novelties are in the five