THE INHIBITION OF REPRODUCTION OF PARASITES BY IMMUNE FACTORS[1]

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First of all, I should like to express my great appreciation for the invitation to give this lecture. I should have considered it a great honor to give any one of this newly organized series, but I feel particularly honored in being selected to give the lecture named after Ludvig Hektoen. Beside my respect for Dr. Hektoen as a scientist, which I share with all of you, I count among the great benefits of moving to Chicago the fact that it has given me some twenty years of his friendship and gracious advice.

Today, I should like to present some of the work on the inhibition of reproduction of the animal parasites in the body by immune factors. I hope to demonstrate some of the advantages of the protozoa and worms for this type of study. The subject has been of interest to me and my co-workers for about 25 years, but it is still a very incomplete picture.

Although immune factors are frequently assumed to inhibit the rate of reproduction of invading organisms *in vivo*, there are only a few instances in which such an inhibition has been unequivocally demonstrated. The difficulty of demonstrating it arises chiefly from two facts. In the first place, most antibody effects result in the fairly rapid death of organisms *in vivo* or their quick removal from the body, or both, whereas an antibody effect on the rate of reproduction takes time and necessitates observations over periods sufficiently extended to demonstrate an inhibition of reproduction. In the second place, where there are no easily recognizable morphological changes due to reproduction, conclusions must be based on changes in the number of organisms. In such cases, a static population is generally interpreted as indicating an inhibition of reproduction whereas such populations may and, especially in malaria, can at times be shown to result from a nicely balanced reproductive rate and death rate of the parasite.

Neither difficulty holds for some of the animal parasites. They can be comparatively easily found in the host, and their reproduction, provided sufficient organisms survive, can be measured directly. Thus, a measure of the reproductive activity of nematode worms can be based on fecundity or the egg-producing capacity of the females. In certain malarial parasites, more or less synchronous reproduction permits a direct measure of the rate of reproduction which is independent of the number of organisms destroyed, and the localization of the parasites to the blood stream allows approximate determinations of the changes of total population by ordinary hematological methods. Various trypanosomes also possess the advantage of being more or less evenly

distributed in the blood, and, although they do not reproduce synchronously, their reproduction can be gauged by the presence of dividing and variable forms due to division and growth.

All inhibition of parasitic reproduction by immune factors must ultimately rest on the impairment of metabolic activities of the parasite. So far, at least two and possibly three types of reproductive inhibition have been found. In the first type, which occurs in the nematodes, immune factors apparently reduce the entire metabolic level of the parasite, including those processes which are involved in reproduction. In the second type, which occurs in some of the malarial organisms, immune factors act similarly but are probably fortified by physiological derangements of the host resulting from the general toxicity of the antigen-antibody reaction. In the third type, which takes place in certain nonpathogenic trypanosomes, immune factors so specifically inhibit reproduction that most of the general metabolic activities of the parasite seem to be carried on without obvious impairment.

1. The Nematodes: Nippostrongylus muris. The first type of inhibition of parasitic reproduction by immune factors is well illustrated by the small hookworm-like nematode, *N. muris*, of the rat. The mechanism of acquired immunity is probably known better in this species than in any other form (see reviews in Chandler (20) and Taliaferro (53, 55)).

The life cycle of *N. muris* is more or less similar to that of many intestinal nematodes and involves both free-living and parasitic stages. The free-living part of the life cycle begins when eggs are passed in the feces of the host. The eggs develop into infective larvae in about a week. The parasitic phase of the life cycle begins with the penetration of the skin by infective larvae and lasts about 2 weeks in non-immune rats. The larvae rarely remain in the skin longer than a day and generally reach the intestine in from 2 to 3 days after migrating through the skin, blood, lungs, trachea, buccal cavity and esophagus. After reaching the upper part of the intestine, the worms develop into adults which measure from 4 to 6 mm in length, and the females generally begin to lay eggs 6 to 7 days after infection. Most of the adult worms are expelled from the intestine approximately 2 weeks after penetration of the skin due to the acquisition of immunity.

Africa (1) and Schwartz, Alicata and Lucker (43) first reported that rats, after recovery from infection with *N. muris*, are relatively immune to a second infection. The cellular and humoral mechanisms of this immunity have been studied from many angles. Suffice it to say here that in non-immune rats, the organisms feed and move freely in the skin, lungs and intestine without deleterious effects to themselves. In the intestine, they feed periodically by piercing the mucosa. In sufficiently immune rats, on the contrary, larvae which penetrate the skin are progressively prevented from feeding and are immobilized and delayed in their migration. Those which are not killed finally reach the intestine but are stunted and are rapidly eliminated. The adult females produce and lay fewer eggs per day and for a shorter period. Schwartz, Alicata and Lucker (43) and Chandler (17, 18) have particularly stressed the retardation of growth and development and the inhibition of egg-laying of the females with-

out, in most instances, any marked lethal effect. Reproduction of the worms, therefore, is markedly inhibited in immune rats.

All of the evidence indicates that the inhibition of growth and egg-laying is the result of an antibody acting as a precipitin. Thus, Sarles and Taliaferro (42) and Chandler (19) were able to transfer the immunity passively. Moreover, Taliaferro and Sarles (59) have demonstrated in their histological studies that the precipitin does not react with the general body surface of living worms but forms precipitates *in vivo* with the excretions and secretions pouring out of the orifices of the parasite, i.e., the mouth, excretory pore and anus. At times, the mouth of the parasite is capped and the whole gut is filled with immune precipitate. Later, after the worm begins to degenerate, antigens apparently diffuse through the cuticle and precipitate forms around the entire worm. All of the phenomena seen in the actively immune animal can be duplicated in the passively immune rat although the various reactions are less intense (60). The immobilizaton of the worms and formation of precipitate can also be duplicated *in vitro* (Sarles, 41).

Antibodies acting as precipitins are probably functional in immunity against several metazoan parasites, including a number of nematodes, ticks and fly larvae (Blacklock, Gordon and Fine (10), Trager (66), and review in Taliaferro (55)). In some cases, they may largely inhibit growth and reproduction as they do in *Nippostrongylus*. In other cases, they probably kill larger portions of the population.

Various hypotheses have been formulated as to how the precipitins act. Blacklock, Gordon and Fine (10), in their work on the larvae of the myiasis-producing fly, *Cordylobia anthropophaga*, first described such precipitates in the gut and around the larvae in the skin of immune guinea pigs. They assumed that the precipitate prevents the assimilation of food and leads to the death of the larvae by mechanically blocking the gut. Chandler (18) considered this expalanation possible but alternatively suggested that the antibodies are anti-enzymes which inhibit the activity of worm enzymes instrumental in digesting and assimilating host proteins. His idea of anti-enzyme antibodies is similar to that of Ascoli's (3) antiblastic immunity in anthrax (*vide infra*). Chandler specifically homologized the anti-enzymes he postulated with ablastin (see later discussion of ablastin, and Taliaferro (53)).

No matter how the antibodies act, it is amply evident that reproduction of such nematodes as *Nippostrongylus* is markedly inhibited. The resulting inhibition of reproduction is not specific, but seems to be simply an expression of a general lowering of the metabolic processes. It is not unlikely that the precipitins act on certain enzymes or enzyme substrates. In either case, they may not specifically inhibit enzyme activity but may simply reduce the effective surface relationship between enzyme and substrate. (See review in Taliaferro (53) for a discussion of the mode of action of precipitins in immunity to nematodes; Dukes (25) and Smith and Lindsley (46) for examples of antibodies affecting bacterial enzymes; and general reviews in Treffers (67) and Sevag (44)).

Immune serums against *Trichinella spiralis* also act as precipitins and have been shown by Oliver-González (35) to be particularly active *in vitro*. Moulder

(unpublished work) has found that anti-trichina immune serum, before it produces any morphological effect on the worms, significantly reduces the oxygen uptake of the larvae utilizing glucose in a Warburg respirometer. This result might have been predicted but is important in view of the more specific inhibition of reproduction to be described later.

2. The Malarial Parasites. The second type of inhibition of parasitic reproduction is found in malaria. It is temporary in nature, and its antibody basis has not been established. The plasmodia allow a particularly accurate measure of the rate of parasitic reproduction.

In 1922, W. H. and L. G. Taliaferro (61) began their studies on differentiating reproduction-inhibiting from parasiticidal factors in immunity. For this work, plasmodia and trypanosomes were selected because, since they are restricted to and more or less evenly distributed throughout the blood stream, the course of their infection can be easily followed by means of frequent blood samples without sacrificing the host. Further progress in this work rested upon developing measures of the rate of parasitic reproduction which are independent of the number of parasitic progeny which die. L. G. Taliaferro (49) pointed out that, for the malarial parasite which has a synchronous asexual reproduction, the length of the asexual cycle and the average number of daughter forms, merozoites, produced by each mature form (suitably corrected, when necessary, for the proportion which grow into sexual stages) give a direct measure of the time necessary for a parasite to grow and produce a given number of progeny and, thus, give a measure of the rate of reproduction. She concluded that the mean number of merozoites produced by segmenting parasites was equal throughout the infection, but she did not make determinations at closely spaced intervals. Boyd and Allen (13) and particularly Boyd (12) showed that the number of merozoites produced per segmenter varies throughout the infection (cf., fig. 2).

The use of the synchronous asexual cycle in conjunction with the number of merozoites per segmenter as a method of measuring the rate of reproduction is well illustrated in infections of *Plasmodium brasilianum* in Central American monkeys as shown by Mrs. Taliaferro and me (62, 63). In this species, it takes three days for one parasite to grow up and produce about ten progeny as may be seen in the top right hand corner of figure 1. The length of the reproductive cycle is also shown in figure 1 by the 3-day peaks in the percent of forms (schizonts) with 5 or more nuclei. This rate of reproduction is generally maintained at a constant rate throughout the infection except when there is a high parasitemia and a sharp parasite decline or crisis terminating the acute rise of the parasitemia.

The parasitemia of an infection with an intense immune reaction at the crisis, as shown by the heavy solid line in figure 1, consisted of an acute rise of the infection (24th to 36th day) with a progressive increase at each segmentation, a parasite decline or crisis (36th to 41st day) with an abrupt decrease, and a developed infection (42nd day on) during which the parasitemia remained at a fairly constant low level.

As may be seen in figure 1, during the acute rise of this infection, the rate of
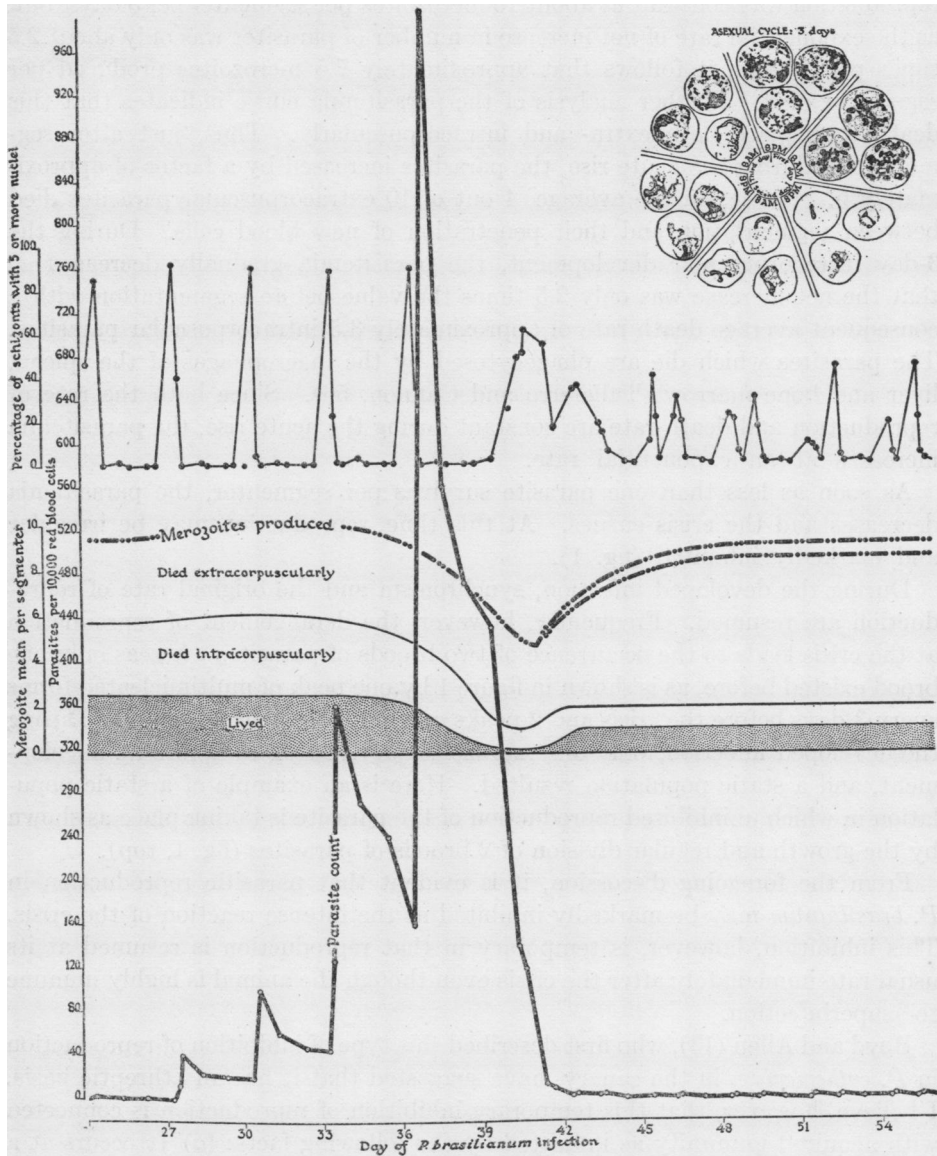
FIG. 1. THE RATE OF REPRODUCTION AND DEATH OF PARASITES DURING THE COURSE OF THE
PARASITEMIA OF *Plasmodium brasilianum* IN A CEBUS MONKEY

The asexual cycle is shown in the upper right corner. The rate of reproduction is indicated by the occurrence of schizonts with 5 or more nuclei in conjunction with the merozoite mean per segmenter (merozoites produced). The number of merozoites which live and develop are computed from the net rate of rise in the parasitemia for each segmentation. The merozoites which die can be divided between those which die intra- and extracorpuscularly.

Note that a drop in the parasitemia (crisis, 39th to 42nd day) occurs when less than one merozoite per segmenter lives and completes its development and that a static population occurs (after the 42nd day) when approximately one merozoite per segmenter lives and completes its development. (Schematic curves from infection shown in (63), graph 3.)

reproduction was constant at about 10 merozoites per segmenter per 3 days, but, as the exponential rate of net increase in number of parasites was only about 2.5 times per 3 days, it follows that approximately 7.5 merozoites produced per segmenter died. Further analysis of the parasitemia curve indicates that this death took place both extra- and intracorpuscularly. Thus, just after segmentation during the acute rise, the parasites increased by a factor of approximately 6. Hence, on the average, 4 out of 10 extracorpuscular parasites died between segmentation and their penetration of new blood cells. During the 3-day intracorpuscular development, the parasitemia gradually decreased so that the net increase was only 2.5 times the value before segmentation with a consequent average death rate of approximately 3.5 intracorpuscular parasites. The parasites which die are phagocytosed by the macrophages of the spleen, liver and bone marrow (Taliaferro and Cannon, 56). Since both the rate of reproduction and death rate are constant during the acute rise, the parasitemia increases at an exponential rate.

As soon as less than one parasite survives per segmenter, the parasitemia decreases and the crisis ensues. At this time, reproduction may be irregular and markedly inhibited (fig. 1).

During the developed infection, synchronism and the original rate of reproduction are resumed. Frequently, however, the derangement of reproduction at the crisis leads to the occurrence of two broods of parasites whereas only one brood existed before, as is shown in figure 1 by one peak of multinucleated forms every 3 days before the crisis and 2 peaks every 3 days after the crisis. During the developed infection, only one merozoite survived to complete its development, and a static population resulted. Here is an example of a static population in which uninhibited reproduction of the parasite is taking place as shown by the growth and regular division of 2 broods of parasites (fig. 1, top).

From the foregoing discussion, it is evident that parasitic reproduction in *P. brasilianum* may be markedly inhibited at the intense reaction of the crisis. This inhibition, however, is temporary in that reproduction is resumed at its usual rate immediately after the crisis even though the animal is highly immune to superinfection.

Boyd and Allen (13), who first described this type of inhibition of reproduction in *P. cathemerium* in the canary, have suggested that it has an athreptic basis. I believe, however, that this temporary inhibition of reproduction is connected with acquired immunity as indicated by the following facts: (a) It occurs at a time of intense agglutination and filtration of the parasites in the spleen and of intense opsonification and phagocytosis by the macrophages of the spleen, liver and bone marrow. (b) It is associated with crisis forms in the circulating blood, i.e., morphologically degenerate forms prevalent at the crisis.

The question immediately arises, however, why is the original rate of reproduction resumed during the developed infection when the animal is still highly immune to superinfection? This may be due in part to a decrease in antibody titer. We are obtaining more and more evidence that immunity is strongest at the parasite decline or crisis and gradually decreases thereafter. It is probably also due in part to toxic effects of the intense antigen-antibody reaction at the

crisis. These effects are manifested by such changes as an irregular fatty change in the liver (Taliaferro and Cannon, 56) and sickness of the monkey such that it becomes sluggish, doesn't eat, and its temperature drops. Indeed, some animals die. This idea is supported by the work of Stauber (47) which indicates that such factors as altered body temperature and host activity markedly derange the asexual reproductive cycle.

In blood-induced infections with *P. gallinaceum* in the chicken, the number of merozoites produced per segmenter and, therefore, the basic rate of reproduction starts at a high level and steadily decreases throughout the acute rise until it reaches a low point at the end of the crisis (fig. 2). Working with this parasite, Moulder and I (unpublished work) have been unable to demonstrate that supposedly immune serum inhibits the oxygen uptake of parasites, either within the red cell or freed from it except in a few cases when the parasites are agglutinated. These negative results may mean nothing because antibodies have only been sporadically demonstrated in this infection. They may, however, be significant and indicate that the toxicity associated with the antigen-antibody reaction is in reality the major factor even though it is not readily demonstrated. Furthermore, the agglutination seen in occasional serums may be similar to the *in vivo* agglutination during the parasite decline or crisis and may represent the direct action of antibodies on the parasites. If so, the inhibition of oxygen uptake would result from a mechanical reduction of the effective surface contact between the parasite and the medium. In so far as the antibody acts directly on the parasite, malaria would resemble *Nippostrongylus* infections in which the precipitin acts on the worm. Thus, agglutination might cause a reduction in surface contact between the organisms and the medium, which in turn might easily cause a generalized suppression of metabolic activity including those processes necessary for reproduction. It may be noted, however, that Sevag and Miller (45) found no diminution of oxygen consumption in *Eberthella typhosa* and pneumococci while agglutinated by immune serum.

The interrelations of parasite reproduction and survival present a fascinating picture and are shown diagrammatically for six infections in figure 2. In this figure, stages of the various infections are shown as if they were of the same length, and detailed parasitemia curves are omitted. In a typical infection by *P. brasilianum*, as contrasted to that shown in figure 1, which had an exceptionally marked immune reaction, the rate of reproduction is maintained at a constant level (approximately 10 are produced every third day) except for a temporary decrease at the parasite decline during some infections, whereas the survival of parasites is constant (around 3) during the acute rise, decreases at the crisis, and then is again constant during the developed infection (although at a lower level—around 1—than during the acute rise) except for sporadic temporary increases during relapses (63).

Infection with *P. lophurae* in the chicken is essentially similar to *P. brasilianum* except that the initial rate is higher (11 merozoites are produced in half the time) and the survival rate (around 4) is higher during the acute rise (W. H. and L. G. Taliaferro, 65, and unpublished work).

In infection with *P. cynomolgi* in the rhesus monkey, the rate of reproduction

progressively decreases during the acute rise until the parasite decline is reached, as shown by a decrease from 15 to 9 in the merozoites produced, then rises and is maintained at an essentially constant level (around 15) during the developed infection. The survival of the parasites is practically 100% at the beginning of the infection but sharply decreases during the acute rise to an extremely low
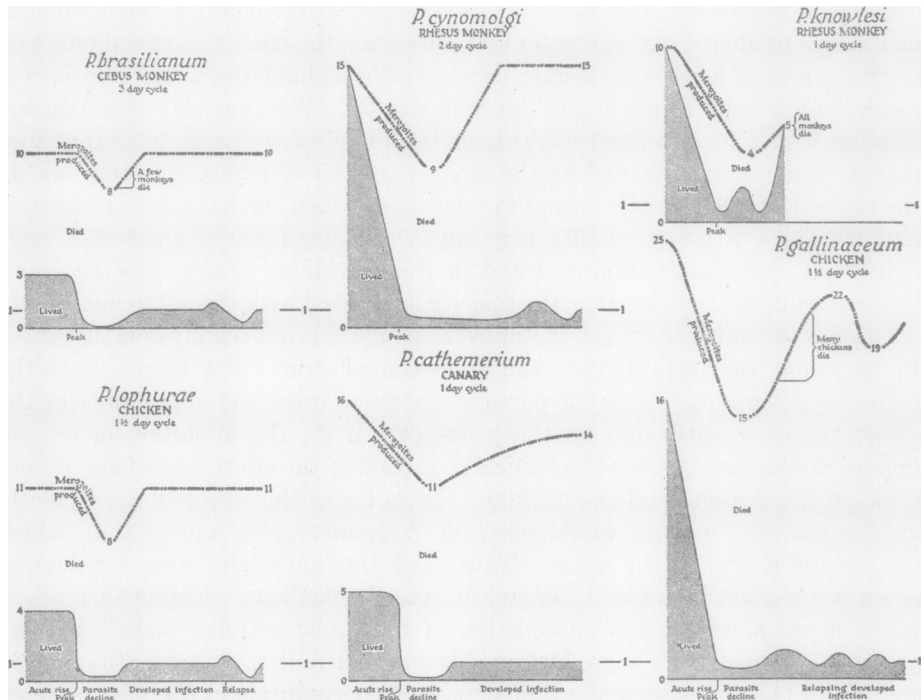

FIG. 2. SCHEMATIC DIAGRAMS OF THE RATE OF REPRODUCTION AND THE SURVIVAL AND DEATH RATES OF SEVERAL SPECIES OF PLASMODIA

The rate of reproduction is shown by the merozoites produced (since the asexual cycle is usually constant) and the death rate is the difference between the forms that are produced and those that live.

Note that, for purposes of comparison, the stages of infection, i.e., acute rise, parasite decline and developed infection, are arbitrarily indicated by the identical intervals in each infection, although they vary over a wide range. (Graphs are based on data of *P. brasilianum* from (63); *P. cynomolgi* from (64); *P. gallinaceum* from (65); *P. cathemerium* from (49), (12), and unpublished work; *P. lophurae* and *P. knowlesi* from unpublished work).

point (less than 1 merozoite) during the parasite decline, and thereafter only temporarily and slightly increases (W. H. and L. G. Taliaferro, 64).

In infection with *P. cathemerium* in the canary, changes in the rate of reproduction are somewhat similar to those encountered in infection with *P. cynomolgi*, whereas the survival rate is somewhat similar to that encountered in infection with *P. brasilianum* and *P. lophurae* except that relapses are uncommon (L. G. Taliaferro (49), Boyd and Allen (13), Boyd (12)). In this infection, we have the anomaly of a generally decreasing rate of reproduction with an essentially

constant survival rate during the acute rise of the infection (Boyd and Allen (13), Boyd (12), and unpublished work by W. H. and L. G. Taliaferro).

In the first part of the fatal infection with *P. knowlesi*, changes in the rate of reproduction and survival of parasites follow the general pattern seen in infections with *P. cynomolgi*, but there is no pronounced crisis and survival of the merozoites increases to 100% just before death (W. H. and L. G. Taliaferro, unpublished work).

Finally, the infection of *P. gallinaceum* in the chicken is somewhat similar to the infection of *P. knowlesi* except that the rate of reproduction is higher throughout (15 to 25), the initial survival of the parasites at the beginning of the infection is less than 100%, and the waves of increased survival are comparatively small (W. H. and L. G. Taliaferro (65) and unpublished work). Although observations on *P. gallinaceum* were made on blood-induced infections in which exoerythrocytic stages are relatively infrequent during the acute rise, the foregoing analysis may be invalidated to the extent that exoerythrocytic stages do occur, particularly after the crisis.

3. Trypanosomes of Rodents: Trypanosoma lewisi. The third, and, to me, the most interesting type of inhibition of parasitic reproduction has so far been demonstrated unequivocally only in infections with trypanosomes belonging to the *T. lewisi* group. It is brought about by an antibody which is so exquisitely specific in action that it inhibits reproduction with no apparent effect on the general vitality, motility, or infectivity of the organisms.

T. lewisi is a relatively non-pathogenic blood parasite of rats all over the world and is transmitted from rat to rat by various species of fleas. It is representative of a large group of trypanosomes which occur in various rodents. All have approximately the same morphology and life cycle, but each is highly specific for its vertebrate host.

The development of the parasitemia in rats following experimental infections induced with infected blood has been described by Steffan (48) and subsequent observers (61, 50, 22, and review in 54). Immediately after the injection of blood there is a prepatent period, which may be of several days' duration or which may be lacking if sufficient organisms are injected. Once the organisms are seen in the blood, their numbers increase and may reach a peak of 300,000 per cmm or more 4 to 7 days later. Once the peak is reached, the parasites may be removed from the blood rapidly in a crisis-like decline or gradually by a series of small decreases. Sooner or later, they reach a somewhat constant number (developed infection) but eventually disappear. Thereafter, the rat is more or less immune to a second infection.

As early as 1899, Rabinowitch and Kempner (37) reported that the blood of rats during the early part of their infection contains many dividing forms whereas later in the infection, it contains only non-reproducing parasites. This conclusion was fully verified by von Wasielewski and Senn (68), Laveran and Mesnil (28), MacNeal (29), Brown (14) and especially by me and my coworkers who applied statistical methods to the problem (Coventry, 22; W. H. and L. G. Taliaferro, 61; Taliaferro, 50). We have found that all mitotic division and

growth is generally completely inhibited by the tenth day of the infection and sometimes earlier.

For these studies and especially for demonstrating an antibody basis, methods of measuring the rate of reproduction are necessary which are independent of how many parasites die. Two basic methods are now in use. The first of these relies upon the occurrence of dividing trypanosomes. The early investigators (37, 68, 28, 29, 14) were content to note the occurrence of dividing forms in blood smears, and this method is still accurate for determining whether or not reproduction is occurring (see 57). Later workers determined the percentage of dividing forms, variously defined, during the course of infection (see Robertson (40) and Krijgsman (27) for pathogenic forms; and Taliaferro and Pavlinova (58) for *T. lewisi*). The second method involving the coefficient of variation was devised by Mrs. Taliaferro and me (61). It depends upon the fact that members of a growing population vary in total length because of young and growing forms, whereas those of an adult population do not. Both methods have certain advantages (58).

The inhibition of reproduction of trypanosomes is brought about by an antibody which I (50) demonstrated in 1924 and later called ablastin (51). Disappearance of the trypanosomes can frequently be associated with a typical lysin which can be complemented with fresh guinea pig serum (51, 52). It may, however, act chiefly as an opsonin (4), possibly, in part, because the rat is deficient in lytic complement.

Ablastin as well as the lysin is specific in action, is precipitated in the globulin fraction of immune serum, and arises after immunization with killed organisms (see 51). As first shown by Regendanz and Kikuth (38), the formation of ablastin is lessened by splenectomy. This antibody has been studied by a number of investigators from several aspects (see review in 54).

Ablastin differs from the lysin in the firmness of its union with trypanosomes (51). If lytic serum is absorbed with either dividing or adult trypanosomes, not only is the lysin removed, but the trypanosomes are sensitized so that they quickly disappear from the peripheral blood when injected into normal rats. In marked contrast, when ablastic serum is treated repeatedly with dividing and adult trypanosomes, ablastin is not removed. This lack of absorption cannot be due to an insufficient number of trypanosomes because the parasites used for absorption are not sensitized, i.e., they are not prevented from dividing when put into normal rats. Actually, however, if sensitization occurred, a blood infection could never be transferred to a normal rat except during the first few days when the organisms are not inhibited by ablastin. This characteristic may differentiate all true ablastic types of antibodies (see discussion of nonabsorbable antibodies in helminth infections, 15, 16, 36, 53).

Recently, Augustine (4) concluded that recovery from reinfection with large numbers of parasites involves only the trypanocidal antibody and that ablastin plays no rôle. He states that the parasites may remain for varying periods without increasing in number even though they are dividing because of a differential susceptibility of the dividing forms to the trypanocidal antibody.

I accept entirely the results of Augustine and consider them to be important extensions of previous findings by Coventry and me. In passively transferring the trypanocidal antibody, I have frequently seen static populations containing dividing trypanosomes. They probably also occur in superinfections because, as Coventry (22) first demonstrated, ablastin decreases in titer as the infection proceeds. The large numbers of trypanosomes which Augustine used would, therefore, be expected to overwhelm the weaker ablastin before it did the stronger lysin. However, Augustine's work can be interpreted as showing that there is no such antibody as ablastin (see Kidd (26), Dubos (24), Becker and Gallagher (8)). In other words, a static population of adult trypanosomes would be brought about by a differential removal of dividing parasites. I cannot accept this view because, in the first place, it is difficult to conceive of an infection in which dividing parasites are removed so completely that they are never seen. In fact, Augustine saw dividing forms in his reinfections although they are not seen in the latter part of initial infections. In the second place, such a view contains an inherent contradiction in that a population, in which adult trypanosomes were

TABLE 1[a]

Oxidative metabolism of Trypanosoma lewisi

REPRODUCTIVE STATE OF TRYPANOSOMES	DIVIDING AND GROWING	NON-DIVIDING ADULTS	DAY OF INFECTION ON WHICH CHANGE OCCURRED
Oxygen uptake μM/10^9 trypanosomes/2 hr.....	45.	62.	5
Glucose utilization μM/10^9 trypanosomes/2hr.	42.	25.	4
Oxygen/glucose ratio......................	1.03	2.78	4
Respiratory quotient......................	0.74	0.91	4

[a] Modified from Moulder (34).

removed as soon as they started to divide or even after they had divided, would not remain stationary but would decrease.

Moulder (32, 33, 34) has begun a study which promises to furnish considerable evidence on the mechanism of ablastic action. In preliminary studies, he has found that ablastic serum has no effect on the oxygen uptake of dividing *T. lewisi* utilizing glucose in a Warburg respirometer (personal communication). Such a result might be expected because of the comparatively short time the serum is acting on the parasite in such experiments. On the contrary, he (32, 34) has obtained positive differences when trypanosomes are observed at different stages in the infection, i.e., when they are rapidly dividing (before the 4th or 5th day of the infection), and after their reproduction has been inhibited by ablastin *in vivo* (after the 5th day of the infection). As shown by the data for the dividing as compared to non-dividing forms in table 1, trypanosomes whose reproduction was inhibited had a higher oxygen uptake, oxygen/glucose ratio and respiratory quotient, and a lower glucose utilization (34).

He points out that the effects of ablastic action *in vivo* are similar to two types of previous findings. Thus, in work on the effect of such inhibitors as urethane, azide and dinitrophenols upon respiration and cell division in yeast cells and sea

urchin eggs, Fisher and Stern, Fisher and Henry, and Krahl and Clowes (see review by McElroy, 30) have found that low concentrations of these inhibitors inhibit cell division with only a slight depression or even a stimulation of oxygen consumption. In addition, Barker, Giesberger and Clifton (see review in 21, 30) have shown that cells not only oxidize substrates for energy but also oxidatively assimilate part of the substrates. In the latter process, more substrate disappears than can be accounted for by its complete oxidation to CO_2 and H_2O, and, therefore, both the respiratory quotient and the oxygen/substrate ratio is low. Low concentrations of such inhibitors as azide and dinitrophenols may inhibit oxidative assimilations without depressing oxygen consumption just as is the case when they inhibit cell division. Under such conditions, the R. Q. rises and oxygen consumption can be largely accounted for by the complete oxidation of the substrate which disappears. Moulder suggests that ablastin may inhibit cell division and growth by inhibiting the oxidative assimilation of glucose in reproducing trypanosomes in a manner similar to the inhibition by azide or dinitrophenol in concentrations too low to reduce the rate of oxygen consumption. Thus, young reproducing trypanosomes oxidize glucose incompletely because they are oxidatively assimilating glucose whereas adult non-reproducing trypanosomes oxidize glucose more completely because the oxidative assimilation has been inhibited.

Moulder (32, 34) further found that, although the degree of inhibition is never great, sodium malonate more markedly inhibits the oxygen uptake of non-reproducing adults than of dividing trypanosomes. This would suggest that the suppression of oxidative assimilation under the influence of ablastin results specifically from the loss of the power to carry oxidation past the succinic acid step in intermediary metabolism. However, Moulder was unable to relieve the malonate inhibition of glucose oxidation with fumarate or to obtain the oxidation of added succinate in either young or dividing trypanosomes. Similarly, Reiner, Smythe and Pedlow (39) reported that succinic acid is one of the end products of aerobic metabolism in this species.

Becker and his associates (7, 9) have found that the reproductive phase of the infection is prolonged when pantothenic acid is withheld from the host's diet or when sodium salicylate is administered per os (8). As a result of these findings, Becker and Gallagher (8) suggested that ablastin is an oxidative enzyme composed of a protein moiety in combination with pantothenic acid. According to them, the apoenzyme is a specific protein which accumulates in the blood during immunization and becomes associated with the coenzyme, pantothenic acid, normally present. They thus explain the effects of pantothenic acid deficiency by the absence of the coenzyme, and the action of salicylate by its combining with the protein in place of the coenzyme.

As far as infections produced by relatives of the lewisi-group of trypanosomes have been studied, they are characterized by the eventual inhibition of reproduction and hence, inferentially by the formation of ablastin. The infection of the mouse with T. duttoni is interesting because, besides an acquired ablastin, the mouse possesses an innate ability partially to inhibit reproduction of T. duttoni

(58). This innate factor cannot be passively transferred and has not so far been demonstrated in a normal rat against *T. lewisi* (52, 54).

4. Related Studies. There are several reports in the literature of immune serums inhibiting the reproduction of bacteria within the body. At the present time, however, such an antibody action has not been definitely established *in vivo* and probably cannot be as firmly established as it has been for some bacteria in the presence of certain dyes or drugs in appropriate concentrations. The difficulty involves the differentiation of a population of viable, non-reproducing cells from one in which active cell division is occurring but is just balanced by the rate of death. In fact, advances in establishing reproduction-inhibiting immune factors may only be possible after we know the metabolic pathways, and the specific action of immune factors on these pathways. I would, however, like to call a few of the more important studies to your attention.

Historically, the most important is the concept of antiblastic immunity which was developed by Ascoli in 1906 and 1908 (2, 3) while working on anthrax in the guinea pig. Parenthetically, it should be noted that I was unaware of this work when I proposed the term ablastin. Ascoli concluded that the protective action of serum is due to the inhibition of certain assimilative processes of the bacteria which prevents germination and is associated with a delay in capsule formation. He further found that this antiblastic principle is nonabsorbable with specific bacteria and does not kill them. Later, when ablastin in *T. lewisi* infections was found to possess these same characteristics, Ascoli believed the antibody he had described to be identical with ablastin (3a). At present, the data seem inadequate to decide whether Ascoli's principle works in the specific way I have described for ablastin. On the other hand, there is not sufficient evidence to disprove it.

Dochez and Avery (23) believed that antiblastic factors occur in antipneumococcus serum which are anti-enzymatic in nature and cannot be explained by agglutination. Later, however, Blake and Barber questioned this. Using single-cell methods, Barber (5, 6) could not obtain any evidence of the inhibition of growth or lengthening of generation time of pneumococci under the influence of immune serum either *in vitro* or *in vivo*. Blake (11) found that immune serum inhibits various metabolic activities of the pneumococcus, but that this inhibition varies with the agglutinin content and disappears after removal of the agglutinins by absorption. The agglutinins act apparently in much the same manner as suggested for precipitins against nematodes in that they decrease the surface contact of bacteria and medium (cf. the negative results on respiration reported by Sevag and Miller, 45).

Campbell (15, 16) has used the property of nonabsorbability to differentiate two types of protective antibodies against the larval form of the tape worm, *Taenia taeniaeformis*, and to homologize one with ablastin. Here again more detailed studies, especially as to the effects on metabolism, will be necessary to prove the similarity or dissimilarity of the two.

Finally, of great interest is the description by Kidd (26) in 1946 of an antibody, produced by rabbits implanted with the Brown-Pearce tumor or injected

with cell-free extracts of it, which is capable of suppressing the growth of the tumor cells *in vivo*. He could observe no microscopic effects of the immune serum on the cells *in vitro*, such as lysis, agglutination or abnormal staining reactions. To analyze such data with respect to reproduction, one prime condition can be satisfied, i.e., mitotic division can be adequately differentiated from cellular destruction just as in the protozoa. The same would be true of tissue cells if the recent suggestion of Medawar (31) is verified. Medawar believes that the destruction of homologous skin grafts in rabbits is due to the action of antibodies which prevent nuclear division in the cells of the grafted tissue.

Conclusions. In conclusion, I should like to stress the fact that most antibodies either kill parasites quickly or effectively aid in rapidly removing then from the body. There are relatively few proven conditions in which antibodies inhibit the basic rate of reproduction of the parasite *in vivo*. Many alleged cases are based on the occurrence of a static parasite population but, in such a population, the members may actually be multiplying and may be being killed by lysis or phagocytosis at the same rate that they are being produced.

There are, however, a few infections in which reproduction of the parasites can be shown to be inhibited. In some of these, the same antibody which kills and aids in eliminating the parasites from the body, also partially inhibits reproduction of the parasites. Thus, a precipitin in worm infections does not specifically inhibit reproduction but probably depresses all metabolic activities— including those on which reproduction rests. In malaria, a somewhat similar nonspecific type of inhibition of reproduction occurs and is probably fortified by the toxicity of the intense antigen-antibody reaction at the crisis which primarily deranges the host's activity and secondarily the parasite's reproductive cycle. The antibody bases for these effects have not been established.

Finally, in infections with *T. lewisi*, ablastin specifically inhibits reproduction of the parasites with no apparent effect on the general activity or infectivity of the parasites. This antibody seems to form no lasting union with the organism. As inhibition of reproduction occurs *in vivo*, the oxidative processes are modified in a manner which suggests that the oxidative assimilation of the substrate is reduced and that in nondividing trypanosomes most of the substrate is oxidized to supply energy for maintenance. Biologically, this antibody is of great interest and may involve mechanisms related to the suppression of cell division in normal development or to the increased cell division in tumors. Unfortunately, the proved occurrence of ablastin in immunity is limited to one group of non-pathogenic trypanosomes, although it may play a rôle in certain bacterial and tapeworm infections and in transplantable tumors.

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