being maintained on the deficient diet containing the antagonist for 10 to 20 days. Vaginal smears were examined daily during gestation for the presence of erythrocytes, the sign that implantation has occurred; all rats were weighed at regular intervals.

Results. Table I shows that placing adult rats the day of breeding on the pyridoxine-deficient diet containing the antagonist resulted in a slight interference with reproduction, evidenced principally by the occurrence of resorptions in 10% of the group. When the rats were placed on this diet 10 to 20 days prior to breeding marked upsets in reproduction were observed. The percentage of resorptions increased directly with extension of the deficiency period, *i.e.* 36% of the rats resorbed after 12 days (11-13) of deficiency prior to breeding, 75% for those averaging 16 days (14-22) deficiency prior to breeding, and 100% of the rats showing the implantation sign, resorbed when the deficiency was extended to 22 days (18-27) before breeding. In the last group it may be noted that a failure of implantation also occurred to a significant extent (29%). The percentage of young born dead was markedly increased (*i.e.* 21%) in the group with 16 days deficiency prior to breeding and a significant decrease in the average weight of the young at birth was also observed. The tendency toward a decreased number of young per litter and decreased weight of the young at birth may be noted in all deficient groups. The normal reproductive behavior of rats supplemented with pyridoxine the day of breeding after 14 days (11-22) of deficiency previous to breeding, showed that the vitamin counteracted all the deleterious effects of the antagonist.

This study adds another dietary condition to those previously found to cause resorption in the adult female rat.[1,2] The rapidity with which the reproductive mechanism is disturbed in this experiment is similar to that previously found in the case of pantothenic acid deficiency. To be noted is the early appearance of the erythrocyte sign frequently observed in this study—on the 9th, 10th, and 11th days of gestation instead of the normal 13th, 14th and 15th days. In the rats not bred, there was an early suppression of the oestrous cycle despite the maintenance of body weight and an early appearance of the characteristic acrodynia on the paws (in 15 days for many rats), indicating the acuteness of the deficiency produced by the antagonist and the importance of pyridoxine for these functions.

Summary. The addition of the pyridoxine antagonist, desoxypyridoxine, to a pyridoxine-deficient diet resulted in marked reproductive upsets when normal adult female rats were placed on the diet only 10 to 20 days prior to breeding. Supplementation with pyridoxine on the day of breeding counteracted the adverse effects of the antagonist.

16454

Morphology of *Hemobartonella muris* as Revealed by Darkfield Microscopy.*

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During the course of studies on *Hemobartonella muris*, we have had occasion to observe the morphology of this organism with the aid of the darkfield microscope. Darkfield preparations of peripheral blood, made at the height of anemia in the rat, have been compared with blood smears made simultaneously and stained with one of the Romanowsky methods.

* Aided by a grant from the John and Mary R. Markle Foundation.

Weinman[1] has presented an excellent summary of the divergent points of view as to the morphology of this organism in the fresh state. These varying opinions are summarized in the following table, which is based upon Weinman's summary of observations from several sources.

I. Mayer, Borchardt, and Kikuth (darkfield and ordinary illumination): Dots only were found—these too few to correspond to the number of stained parasites.[2]

II. Ford and Eliot: Nothing suggestive by direct lighting either in hanging drop or unstained film—motionless parasites visible in darkfield.[3]

III. Dinger: Parasites present in darkfield—animated by Brownian movement.[4]

IV. Lauda: Parasites in darkfield are motionless.[5]

V. Bayon[6] and Perez[7]: Slow and sinuous motion of the organisms was observed in the red cells.

VI. Noe and Paez[8] and Rordorff[9]: Report rapid motion of the organisms.

VII. Levi[10]: Observed innumerable refractile granules, the majority free in the plasma, which showed 5 to 10 minutes lively motion, not of a Brownian type and then became quiescent.

In our own work, we feel that we have amply confirmed the variation in morphology reported for this organism as seen in the stained smear. The extremely fine granular forms reported by Vasiliu,[11] the dumbbell shaped organisms, the flame shaped diplococcoids, rods, arrangement in short chains, and indeed the entire range of morphological variation described by previous workers has been observed.

We felt that further use of darkfield examination of the blood of parasitized rats might be valuable. First, we believed that it might give us some conclusive evidence as to the position of the organism relative to the red cell; and second, that it might provide additional data as to motility of *H. muris*

Repeated examinations of peripheral blood of splenectomized animals with the darkfield microscope have been made, and, simultaneously, the same blood has been examined in the stained smear. Comparison of these two types of preparation has convinced us that there is, sometimes, a marked disparity between the number of organisms that can be seen in the stained preparation, and those seen by darkfield examination. Our studies have convinced us that *Hemobartonella muris* in the fresh preparation exhibits a motility which, though limited, is definitely in excess of Brownian movement. Use of darkfield preparations has further convinced us that this organism may be both intracellular and epicellular.

In reference to the first observation noted above, namely the disparity in number of organisms in the darkfield preparation and stained smear, some remarks are pertinent. We have noted that organisms seldom seem as numerous in the darkfield preparation as in one stained with Wright or Giemsa stains. When a darkfield preparation is made and immediately viewed, the most striking element is the granular form of the organism. These granular forms are observed in the cells and free in the plasma. Some difficulty is at first experienced in differentiating the organism from hemaconia. A little practice, however, enables one to make this differentiation without much difficulty. The hemaconia are more constant in morphology, they are larger and they are more refractile than is the gran-

1 Weinman, David, *Transactions of the Am. Phil. Soc. N.S.*, 1944, **33**, 243.

2 Mayer, M., Borchardt, W., and Kikuth, W., *Arch. f. Schiffs-u. Tropen-Hyg.*, 1927, **31**, 295.

3 Ford, W. W., and Eliot, C. P., *J. Exp. Med.*, 1928, **48**, 475.

4 Dinger, J. E., *Nederl. tijdschr. v. geneesk.*, 1929, **73**, 1910.

5 Lauda, E., *Patho. Mikroorg.*, 3rd ed., 1930, **8**, 1073.

6 Bayon, H. P., *J. Trop. Med.*, 1928, **31**, 29.

7 Perez Aro, A., *Vida nueva*, 1933, **32**, 51.

8 Noe, J., and Paez, R., *Rev. Inst. Bact. de Chile*, 1930, **1**, 20.

9 Rordorff, R., *Gior. de bateriol. e immunol.*, 1933, **11**, 351.

10 Levi, M., *Haematologica*, 1931, **12**, 1.

11 Vasiliu, N., *Compt. Rend. Soc. de Biol.*, 1929, **102**, 173.

ular variety of *H. muris*. These criteria allow one to differentiate the granular form of *H. muris* from hemaconia; differentiation, of course, of the longer form of *H. muris* from hemaconia presents no difficulty. Some practice in observation of darkfield preparations of fresh blood enables one to make out especially in the red cells, longer forms of *Hemobartonella muris* which are very thin.

We feel it entirely likely that all of the morphological variations which are present in the stained smear are present in the fresh preparation. Because of lack of color contrast and the extremely delicate character of the organisms, these morphological variants are difficult to observe in fresh preparation. In the larger red cells particularly, we have observed a marked variation in the shape of the organisms found in relation to a single red blood cell. In the stained preparation, not only is the eye aided by the color contrast but the organisms, as might be expected, appear to be considerably larger than in the unstained preparation.

Motility is most marked when the darkfield preparation is first observed; it abates as time progresses. Motility of the forms free in the plasma, which is very marked when a preparation is first viewed with the darkfield microscope, diminishes as the fibrin network is formed. After the formation of fibrin, active motility is seldom observed. Whether this is due entirely to the fibrin formation or due simply to the age of the preparation, we are unable to say at the present time. The motility exhibited in the plasma is, we feel, unquestionably directional in character. Organisms traverse the field from one portion to another past intervening cellular structures in a rather slow but unidirectional movement.

Of considerable interest is the activity of those organisms which are related to the red cells themselves. We have observed single parasitized red cells over a relatively long period of time and have noted that those organisms which appear to be intracellular do not hold one fixed position but, on the contrary, may move about quite freely. At times, such parasites will be distributed over the extent of the entire cell; at times, they will all be arranged about the cell periphery. Organisms so related to the cell are often in constant motion. The motility of elongate forms is less likely to be vigorous than is the case of the granular ones. In those organisms which show considerable length, the slow sinuous motion reported by Bayons and by Perez has been repeatedly observed. We have also seen a bending of the elongated organisms in their central portion followed by extension to their full length. This alternate bending and extension appears to play some role in the propulsion of the organism across the field.

We have observed parasitized cells for prolonged periods without observing an organism to leave the confines of the cell. It is difficult for us to understand how such forms could be simply epierythrocytic as has been reported to be true in the case of *Bartonella bacilliformis*.[1] We have frequently seen forms which obviously occupied a position on the exterior of the cell, and such forms have been seen to separate from the cell and again to become free in the plasma. We are in agreement with McCluskie and Niven[12] that *H. muris* is found both on and in the red cell of the rat.

Summary. Darkfield examination of the blood of rats parasitized with *H. muris* shows that organism to have a motility which, though limited, is directional in character. Motility of the organisms in the plasma is diminished rapidly as the preparation ages. The forms seen in darkfield preparation are not limited to the granular variety; numerous variations in morphology are present and probably the entire morphological range as can be seen in the stained film. Our observations lead us to conclude that the organisms are both intracellular and epicellular in position in relation to the red cell, as well as being free in the plasma.

[12] McCluskie, J. A. W., and Niven, J. S. F., *J. Path. and Bact.*, 1934, **39**, 185.