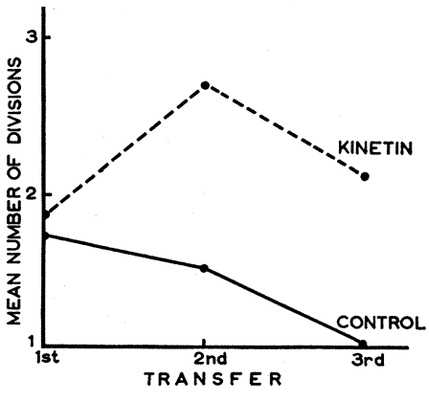

Fig. 1. Cell division in *Paramecium caudatum* under suboptimal culture conditions. The influence of kinetin on division frequencies after each of three daily transfers to new medium.

kinetin, cell division frequencies were also increased, but on a consistently lower level. Because of this lack of reproducibility, our earlier data have been reanalyzed, and these, together with the preliminary results of additional experiments, are presented here.

The results reported in the earlier paper (*1*) were of daily division rates based on the mean of three successive transfers. With 1 mg of kinetin per liter, the ratio of mean divisions in kinetin: mean divisions in control medium ($\bar{k}/\bar{c}$) was 1.45 for the 3-day period, which is highly significant. But when, subsequently, division frequency was recorded for each day separately, an entirely different picture was obtained. Figure 1 shows the original results, separated into the three daily transfers. Kinetin significantly increased the division rate even after the first transfer, but only by 10 percent. The high $\bar{k}/\bar{c}$'s of the following two days (1.78 on the second and 2.19 on the third) were due to two factors: (i) a rising division frequency in the kinetin-containing medium over the 3-day period and (ii) a drop in cell multiplication in the control medium, particularly after the second transfer.

Division of the protozoa after transfer is known to depend on such variables as the amount of food in the new medium, the condition of the transferred animal, the number and kind of bacteria transferred along with the paramecium, and other factors. Depression of division after transfer from stock solution was discussed in an early paper by Chejfec (*2*), while a detailed analysis of the effects of varying culture conditions may be found in a book by Wichterman (*3*).

Multiplication frequencies in our original experiments were low—never more than two divisions in 24 hours. One may assume that some factor necessary for division was suboptimal in these cultures and that this factor was increasingly diluted during the first three transfers. Unfortunately all experiments were terminated after 3 days, and no data are available on whether or when an equilibrium may have been reached.

The following series of tests were conducted a year later, with a new clone of *Paramecium caudatum*, on infusion made from a new batch of hay, and with two new samples of kinetin (California Biochemicals and Waldhof). In these cultures, division frequency per 24 hours was always above 2 and sometimes as high as 5. The experimental procedure was the same as in the previous tests, but daily transfers were made for 6 days. The data given in Fig. 2 are the means of results from ten separate series of tests. In these experiments there was no significant drop in division frequency in the control group. The daily k/c's varied from 1.07 to 1.11, each k being significantly above c at the 5 percent level. Thus even under near-optimal conditions of culture, kinetin could shorten the interdivision period—in these experiments from an average of 8.4 hours in controls to an average of 7.7 hours in kinetin. The constancy of the k/c's over the 6-day period indicates that kinetin does not accumulate in the protozoan, either to increase its stimulatory effect with time or, conversely, to reach an inhibitory level.

A subsequent experimental series was designed to ascertain the presence—or absence—of a stimulatory effect of 1 mg of kinetin per liter during the latter half of the interdivision period. Sixty paramecia were isolated from stock solution, randomized, and divided into groups of 30. One group was placed (singly) in control medium, the other in kinetin-containing medium. After 5 hours' incubation, the number of individuals in each group was recorded. This series was replicated five times. Generation time in these cultures (as ascertained by routine examinations) was approximately 12 hours. However, at the end of the 5-hour period only 5.33 percent of the controls had divided, while 12.67 percent of the paramecia transferred to kinetin-containing medium had completed cell division. The t-value for the difference between divisions in control and divisions in kinetin medium was found to be significant at the 0.01 level. These data indicate that kinetin may be of aid in overcoming the lag period following transfer from stock culture to depression slide, perhaps again by providing or substituting for some factor that is limiting under these conditions.

Finally, it should be pointed out that several samples of kinetin have been found to become toxic after about a year, even though they have been tightly closed and refrigerated. Also, bioassays of equal doses of California Biochemicals and Waldhof kinetin have given dissimilar results, the German preparation having a lower stimulatory action. Lack of uniformity in the effects of kinetin on microorganisms has also been reported by Braun (*4*), who ascribes some of the variability to an influence of high levels of amino acid and trace metal on the action of kinetin (*5*).

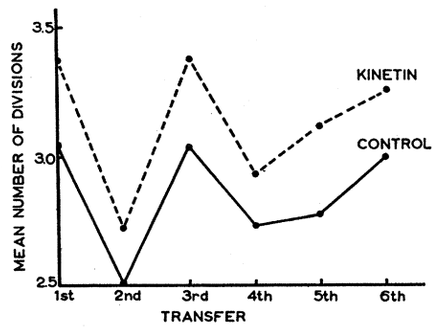

Fig. 2. Cell division in *Paramecium caudatum* under favorable culture conditions. The influence of kinetin on division frequencies after each of six daily transfers.

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5. These studies were supported in part by the Anna Fuller Fund.

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Detection of an Anaplasma marginale Antibody Complex Formed in vivo

Abstract. A naturally occurring anaplasma-antibody complex was detected by exposing erythrocytes of infected cattle to fluorescein-labeled bovine antiglobulin. This technique revealed not only the classical marginal bodies but also the initial *Anaplasma* bodies in the erythrocytes of acutely infected animals. Singly occurring initial bodies were observed in the erythrocytes of healthy carriers.

Detection of an antigen in Weller and Coons' indirect fluorescent antibody method is accomplished by exposure in vitro to unlabeled antiserum derived from naturally infected animals or to antiserum produced by rabbits after inoculation with the antigen, and subsequent treatment with fluorescein-labeled antiglobulin (*1*). A modification of this technique was described by Mellors (*2*), who demonstrated histological

sites of nephrotoxic antibodies with fluorescein-labeled antiglobulin.

In the present procedure used to detect *Anaplasma marginale*, the antigen-antibody reaction is a naturally occuring process within the blood-vascular system of infected animals. To demonstrate this reaction in vitro, thoroughly washed erythrocytes from infected cattle were exposed to the fluorescein-labeled bovine antiglobulin.

Normal bovine globulin was extracted from the serum of a normal calf, according to the method described by Dubert (*3*), which involves the precipitation of globulins with methanol. The antiglobulin was produced by injecting a rabbit three times a week for 3 weeks with 0.5 ml of normal bovine globulin in increasing concentrations (20, 40, and 80 mg). The globulin was precipitated from the antiserum and conjugated to fluorescein isothiocyanate, according to the method of Riggs *et al.* (*4*). To minimize nonspecific fluorescence, the conjugated material was absorbed with powdered rabbit liver prior to use. The technique of fixation and staining of blood films with labeled bovine antiglobulin was similar to that used by Ristic *et al.* (*5*) for detection of *A. marginale* by means of specific fluorescein-labeled antianaplasma antibody.

By the method described above it was possible to observe *A. marginale* growth forms, including marginal and initial bodies (*6*). It was also possible to detect a single initial body occurring within erythrocytes of carrier animals. As viewed with a microscope by ultraviolet light, the initial bodies appeared as punctiform, brilliant foci scattered throughout the erythrocytes of acutely infected animals (Fig. 1) or appeared singly within the erythrocytes of carrier cattle (Figs. 2, 3). The classical *A. marginale* occuring in acute infections appeared as brilliant yellow-green, sharply defined, round bodies (Fig. 1). In contrast, the erythrocytes were clearly seen as grayish-green background structures.

With this technique it was possible to demonstrate the presence of initial bodies not usually observed by conventional staining procedures. This is apparently due to the formation at the site of the organism of a specific, fluorescent-complex aggregate of sufficient size to be observable by microscope. While further evaluation of this technique with regard to its accuracy in detecting anaplasmosis carriers is needed, the principles upon which it is based offer a means of developing a serological test capable of revealing the organism rather than serum antibodies in the blood of anaplasmosis carriers.

It seems reasonable to believe that by the technique described above, other organisms capable of invading the blood-vascular system could be detected. In addition, this method may prove useful in demonstrating *Vibrio fetus, Trichomonas fetus, Leptospira* species, and other microorganisms which, after an acute stage of infection, may persist for long periods in extravascular localities of the body. In these areas complex cell systems apparently are capable of producing antibodies in local tissue that, when combined with specific antigens, offer the prerequisite for carrying out the technique described (*7*).

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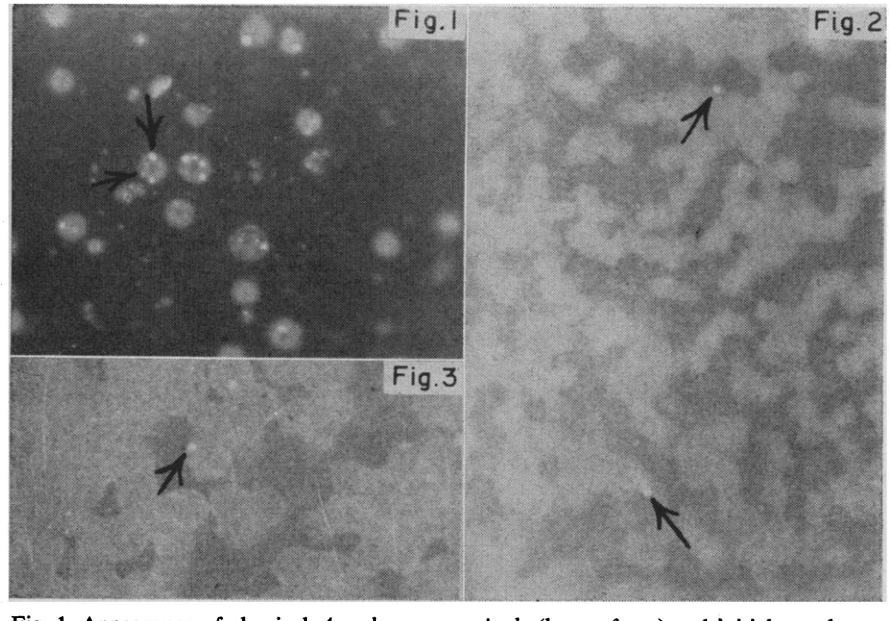

Fig. 1. Appearance of classical *Anaplasma marginale* (larger form) and initial anaplasma body (smaller form) in the blood film from an acutely infected cow after staining with fluorescein-isothiocyanate-labeled bovine antiglobulin (about × 600). Figs. 2 and 3. Erythrocytes of two anaplasmosis carriers, stained with fluorescein-isothiocyanate-labeled bovine antiglobulin. Note the presence of three initial anaplasma bodies (about × 900).

Significance of the Presence of Exchangeable Magnesium Ions in Acidified Clays

Abstract. Magnesium ions (Mg^{++}) were shown to constitute a substantial percentage of the total exchangeable cations in acidified clay samples from various sources. This finding helps in solving several problems of long standing in the fields of soil chemistry, soil formation, and geochemical weathering.

Many investigators have concluded that the acidity of naturally occurring soils and artificially acidified soils and clays is due mainly to the presence of exchangeable Al ions rather than to exchangeable H^+ (*1*). It is postulated that after the exchange of the basic cations with H^+ ions, the H^+ ions disappear from the exchange positions and are replaced by Al ions which are either a part of the interior of the crystal lattice (tetrahedral or octahedral positions) or part of free $Al(OH)_3$ and Al_2O_3 which are present in the soil or clay as impurities. Several investigators, however, were aware that the H^+ ions are replaced not only by Al ions but also by Mg^{++} ions (*2*), but the factors affecting this replacement and its significance were only studied recently. The present report is a result of this study.

Briefly, the relative amounts of Mg^{++} and Al^{+++} ions were found to depend on the total MgO and Al_2O_3 contents and the crystal structure of the acidified minerals and on the nature of the acidifying solution and the technique of acidification.