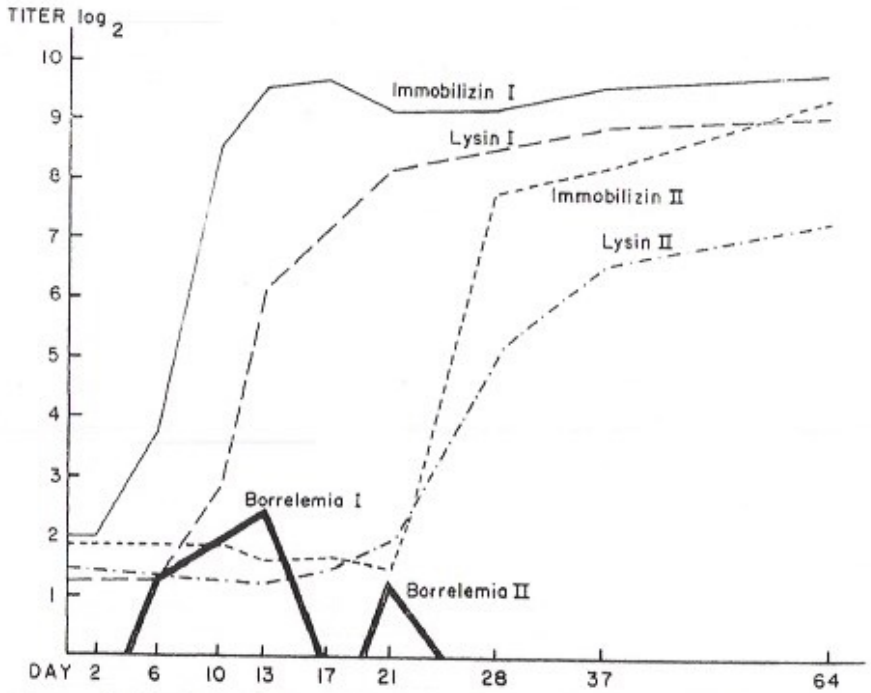

FIGURE 4. Serologic data, patas monkey *(Erythrocebus patas)* infected with *Borrelia parkerii*.

Immobilizines were said to be of small molecular size (449) and are related to β and γ globulins (136). They were observed also in the heavy (19S) IgM (268). Immobilizines can be found in the serum long after the infection has subsided (268, 449, 450) and may be directed against a specific phase of the causative agent (598). These antibodies are present also during the latent phases of the disease. They are strain specific (697). Levaditi *et al.* (450) expressed the opinion that antibodies such as immobilizines may induce phase variation in borreliae.

The borreliolytic activity of the serum of infected man and animals appears to be identical to antiborrelial cytolysin and borreliocidin (262). It was considered inconstant in guinea pigs infected with *B. hispanica* but in man it persisted for nearly a year

(572). Toyoda (688) had the same experience. Ranque *et al.* (597, 599) found it highly specific, whereas complement fixation test, fluorescent microscopy, and skin tests showed cross-reactions. The cardiolipid hapten and group proteins are, however, common to related organisms.

Toyoda (688) and Wolstenholme and Gear (737) described complement fixing antibodies in relapsing fever which will be further discussed in the chapter on Laboratory Diagnosis.

Immunity to borreliae has been termed a premunition-like phenomenon by Geigy and Burgdorfer (307). The studies of Chamsa (153) led him to the conclusion that this premunition is strain specific.

Loewy (455) correlated the periodic changes of body temperature during relapses ending with crisis, and the lesions often observed in the nervous system in the course of the disease, principally when treatment has been started late. Therefore, he concluded that anaphylactic phenomena play a role in relapsing fever.

Little has been said about natural antibodies in borreliosis. Weichbrodt (719) questioned whether they may not be present in the cerebrospinal fluid. Such antibodies, or the lack of some physiologic capabilities of certain *Borrelia* strains, could explain why not all borreliae invade the central nervous system.

Staining

Borreliae have an affinity for acid dyes, whereas many other bacteria prefer basic dyes (720). Nevertheless, borreliae can be stained with practically any aniline dye (132). Azur-eosine and related stains of Leishman, Giemsa, May-Grünwald, Romanowski, Wright, and their combinations are favored for staining blood films from patients and animals.

Du (246) described a simple and effective method feasible also for the staining of thick blood smears. The slides are dehemoglobinized with 6% acetic acid in 95% ethanol, rinsed, and then stained with carbolfuchsin for 1 minute.

Pampana (561) stained thick drops with a 2% methylene blue B extra solution in distilled water, to which 4 ml formol and 10 ml glacial acetic acid were added after filtration. Methylene blue was used also by Simons (648) who mixed 1 ml saturated

methylene blue solution in physiologic saline with 2 ml 10% sodium taurocholate in saline, added 2 to 4 loopfuls of this mixture to an equal volume of blood, then made smears with it on microscope slides. This method can be used also for the examination of citrated blood which first has to be centrifuged and then the sediment can be examined.

As most workers do who use routine blood stains, Coles (196) also recommended prolonged staining but employed orange tannin for differentiation after the coloration. Vago (696) and Young (743) recommended mercurochrome. The latter employed concentrated aqueous mercurochrome for 3 minutes, followed by concentrated aqueous methyl violet. Our group (264) applied 1% crystal violet for a few seconds after staining according to Wright. Other combinations, such as the use of saturated alcoholic or aqueous solutions of a basic dye followed by an acid dye in 30% alcohol (gentian violet and acid green, or brilliant green and acid fuchsin) were recommended by Weiss (720). Levine (451) used careful fixation of airdried smears, first with acid-free chloroform, then with acid-free absolute ethanol. Fuchsin was recommended for staining.

Fluorescent antibody studies of borreliae were made by Coffey and Eveland (184) who found them superior to the immobilizine and lysin tests. Maestrone (463) used the fluorescent antibody method for leptospiral and viral antigens in formol-fixed tissues that is applicable also to borreliae. The tissues are re-fixed with acetone for 5 minutes, dried at 37° C, exposed to ammonia vapors for 2 days at 37° C, or acted upon with 1% ammonia for 3 to 5 minutes. Sodium bisulfite, 25% for 5 minutes, may be substituted. The slides are washed with 3% Tween 80 containing saline buffer pH 7.2, blotted dry, reacted with rabbit anti-*Borrelia* serum or globulin, then, after washing with buffered saline, sandwiched with a fluorescein isocyanate or rhodamine labeled anti-rabbit serum, and mounted in glycerol. A slight shrinkage is usually apparent.

Silver impregnation methods are used for the visualization of borreliae in tissues. That of Krajian gives excellent results and will be described in the Appendix in detail.

The methods of choice will be discussed in the chapter Laboratory Diagnosis.

Culture Methods

In Vitro

Noguchi (544, 545) succeeded in growing *B. recurrentis* and *B. duttonii* in a rabbit kidney-ascitic fluid medium, under liquid paraffin seal. Maximal growth was observed in 7 to 9 days. First short, then longitudinally dividing forms were seen. Noguchi succeeded in passing the Koch strain 29 times over a period of about 6 months in this medium but others (262, 513) were not successful in attempts to culture borreliae from the blood and organs of patients using this medium. Others (658) had better results with laboratory strains. Kligler and Robertson (414) pointed out that the medium should be slightly alkaline. These authors used ascitic fluid, horse or rabbit serum, 1% peptone broth, or egg albumin solution. Moroder (504) employed a mixture of inactivated rabbit or horse serum with 2 to 5 parts of physiologic saline, and covered the cultures with liquid paraffin. Granules still present in old cultures were infective for mice. Li (452) dispersed the yolk of one egg in 400 ml physiologic saline and added egg white. After coagulation, liquid paraffin was layered over the slants. One or 2 drops of citrated blood were put into the supernate when transfers were made. Chorine and Crogue (169) also used blood. Their medium contained peptone water, fresh rabbit serum, Tyrode's solution (which could be omitted), and laked or defibrinated human blood. It took 7 to 8 passages to establish the slowly growing strains. Others (44) were not successful with the medium of Chorine and Crogue.

Wolman and Wolman (736) prepared their medium by adding 10 ml human ascitic fluid to 1 ml coagulated egg albumen. An equal volume of buffer pH 7.8 and 2 volumes of 1% dextrose were added. After covering with liquid paraffin, the mixture was held at 56° C for 1 hour each on 3 consecutive days. *B. recurrentis* lived and multiplied in this medium for 8 months but lost its virulence after a year. Krylova (426) had good results with a modified Wolman procedure.

It should be noted that most authors who succeeded in growing borreliae *in vitro* did so at 28° to 30° C, or at even lower temperatures.