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Studies on *Ehrlichia canis* (syn. *Rickettsia canis*).

By P. H. BOOL.

“La Rickettsiose et la Leishmaniose ont été les maladies les plus fréquentes et les plus graves du chien” (SERGENT, Algeria 1945).

“Whether they are true micro-organisms must await further study” (TOPLEY and WILSON 1947).

After the first description of a rickettsial disease in the dog by DONATIEN and LESTOQUARD (1935) and the investigations instituted by them (1936, 1937, 1940) on the cycle, the carrier, and the existence of premunition, in the years following the literature was restricted to reports concerning the geographical incidence of this organism and to clinical observations (HENNING 1956, LEVADITI et al. 1943).

Isolation of an *Ehrlichia canis* strain from some specimens of *Rhipicephalus sanguineus*, found on Aruba, Netherland Antilles (BOOL and SUTMÖLLER 1957), gave the opportunity of obtaining supplementary data. Although the investigations concerning culture and serological reactions have not led to a positive result, yet attention may be drawn to this interesting subject with its many unsolved problems.

Nomenclature:

MOCHKOVSKI (1937) combined the “bodies of Kurloff”—appearing in monocytes in guinea-pigs—with *Rickettsia canis* into a new subgenus “*Ehrlichia*” and defined *E. canis* as type. MOCHKOVSKI (1945) subsequently introduced “*Ehrlichia*” as a genus. Although his study in 1937 did not clarify the nature of the “bodies of Kurloff”¹ nor show any relationship to *E. canis*, nevertheless the genus *Ehrlichia* is accepted and used in the literature (COLES 1953, GORET et al. 1955, PHILIP 1953). As the old nomenclature *Rickettsia canis* incorrectly suggests a close relationship to the classic—for man pathogenic—rickettsiae (WEYER 1955), we also give preference to the name “*Ehrlichia*”, notwithstanding the fact that some obscurity exists regarding its origin.

In this connection it is important to realise that DONATIEN and

¹ The opinion that these “bodies of Kurloff” are to be considered as infective agents, has received little support since 1937. See the extensive studies of LOUWERENS (1952).

LESTOQUARD (1936), in a comparative investigation of *Rickettsia conori* and *E. canis*, reach the conclusion that: "Le seul point de ressemblance entre ces deux protistes est le fait d'être transmis par la même tique." The arguments which induce DONATIEN and LESTOQUARD to group *E. canis* with the rickettsiae are, in addition to some morphological resemblances, the existence of a long-lasting premunition, the fact that under natural circumstances a tick transmits the disease, and that during the course of the disease generalized skin eruptions and nervous symptoms appear.

ROUSSELOT (1953) in his excellent "Notes de parasitologie tropicale" has proposed the genus "*Donatienella*" for micro-organisms which are of uniform shape and size and which are found in monocytes in the blood of animals. However, priority must be accorded to the genus *Ehrlichia*.

Experimental data:

In the experiments with *E. canis*, carried out in Utrecht, the characteristics of the micro-organism and the reactions of the test animals were studied. This study was partly a confirmation of the experimental results found in the literature. In addition, however, data were obtained which were in opposition to these findings. Particular attention will be given to the divergent results.

Twelve native mongrel dogs of various ages were infected with *E. canis*, one by subcutaneous inoculation of 4 *Rhipicephalus sanguineus* females triturated with broth, the others by subcutaneous or intravenous injections of infected dogs' blood. Blood smears were made from a small scarification in the ear. To establish the presence of *E. canis*, the first drop of blood was examined, since it contains a significantly larger number of leucocytes than the following drops, which are, on the other hand, used for differentiation of the leucocytes.

All slides were stained with Giemsa.

Since it has been claimed that *E. canis* can be stained only for a short period after the death of the host, tissue smears were made every hour from a portion of a lung, kept at 20°C in a moist atmosphere. It was found that even after 23 hours the *E. canis* was as readily stained as in a preparation from a fresh organ. After 27 hours, however, staining was relatively ineffective.

Virulence of the E. canis strain.

Of the 12 infected dogs 2 animals died, one after 7 weeks as a direct result of infection with *E. canis*, the second after 9 months, after a splenectomy with clinical and parasitological relapse and

two re-infections. From the liver of the latter dog *Salmonella dublin* was isolated; the microscopic picture of the liver showed interlobular necrotic foci similar to those appearing in a *Salmonella* infection. As the necrotic foci were of a recent date the salmonellosis should be considered as a secondary infection.

Babesia canis and *Hepatozoon canis* were never found in the blood and tissue smears; moreover, blood cultures in liver-broth showed the absence of bacteriological infections.

Piroplasmosis canis is unknown in the Netherlands, with the exception of a rare imported case.

ROUSSELOT (1953) points to the fact that the virulence may differ very much. The strain used in Utrecht would appear to have a low virulence, because of the few fatal cases resulting. A definite statement cannot be made, however, due to the lack of comparable experiments. According to NEITZ and THOMAS (1938) in South-Africa, all the 30 dogs infected with *E. canis* succumbed; their statement "in many of the dogs used, *Piroplasma canis* appeared either as a result of a relapse or as a result of the infective blood of the donor" points to the complicating factor which *Babesia canis* possibly had on the course of the disease. ROUSSELOT (1953) states that in French Soudan 50% of the experimentally infected dogs died, most of them after several relapses and the manifestation of latent piroplasmosis. The splenectomies performed by him always resulted in death of the animals, but only after *Babesia canis*, and *Hepatozoon canis*, in addition to *E. canis*, had appeared in the bloodstream: "si bien qu'on ne peut rapporter la mort qu'à l'association rickettsiose-piroplasmose sans pouvoir déterminer la part de chacun des agents en cause". DONATIEN and LESTOQUARD (1937) also describe the serious course of the disease occurring in dogs latently infected with piroplasmosis and subsequently inoculated with *E. canis*.

In order to determine whether the *E. canis* strain used in Utrecht is actually of a low virulence, or whether the *E. canis* infection in general is not a serious disease, as GILLAIN (1945) claims, it would be necessary to perform comparative experiments with other *E. canis* strains in a region where no dog-piroplasmosis occurs.

The *clinical picture* and the autopsies of the test dogs have previously been described (BOAL and SUTMÖLLER 1957). The clinical progress of the infections has not confirmed the statement of CARMICHAEL and FIENNES (1942) who propose a division into a cutaneous, a septic, and a nervous form, so that we agree with the opinion of MALHERBE (1948) that these phases occur at the same time.

In order to determine whether *congenital* infection is possible, blood and tissue material, in which no *E. canis* could be found microscopically, of a 4-day old pup born of an infected mother were injected into a dog. This animal showed no reaction and on re-infection proved to be normally sensitive.

The existence of a long-lasting *premunition* (DONATIEN and LESTOQUARD 1937) has been confirmed. In the case of one dog, 15½ months after infection it was still possible to transmit the *E. canis* with the blood. The splenectomy performed 8 months later on this animal did not cause a clinical relapse, thus indicating that apparently the animal had freed itself from the parasite. Since, however, no regular microscopic examination of the blood was made after the operation, an other test dog was infected 2 months later—thus 25 months after the original infection—with 15 c.c. of blood i.v. After a somewhat longer incubation period (26 days) the animal showed high fever and *E. canis* was found in the peripheral blood. It appears from this that a condition of *premunition* had lasted for at least 23 months.

Changes in the blood:

The hypochromic *anaemia* assumed a very serious form in all the test dogs; as shown in figures 1 and 2, the rapid fall of the haemoglobin content starts already in the first days of fever and only returns to normal after 3 to 4 months. During this period normoblasts and symptoms of polychromasia and anisocytosis are always present. The number of erythrocytes may fall to 2 million. In addition to a long coagulation time of the blood, there is also an increased sedimentation rate which returns to its normal value after the clinical attack, in spite of the continued presence of anaemia. The number of leucocytes decreasing slightly in the beginning, rises during the first phase of the fever to 15.-20.000.

It is remarkable that the monocytosis, which is repeatedly emphasized in the literature, did not appear in any of the test dogs. This lack of monocytosis can again be regarded as an indication that the experimental infections have generally been made with a combination of *Babesia canis* and *Ehrlichia canis*. In the case of piroplasmosis in dogs the monocytosis is indeed very pronounced; REUSSE (1954) finds for 21 test animals an average of 23% monocytes at the height of the piroplasmosis as compared to an average of only 4% for healthy dogs.

In a study on the causative agent of tick-borne fever in sheep, FOGGIE (1951) mentions that "*E. canis* invades the large lymphocytes—described in the French literature as monocytes". CARM-

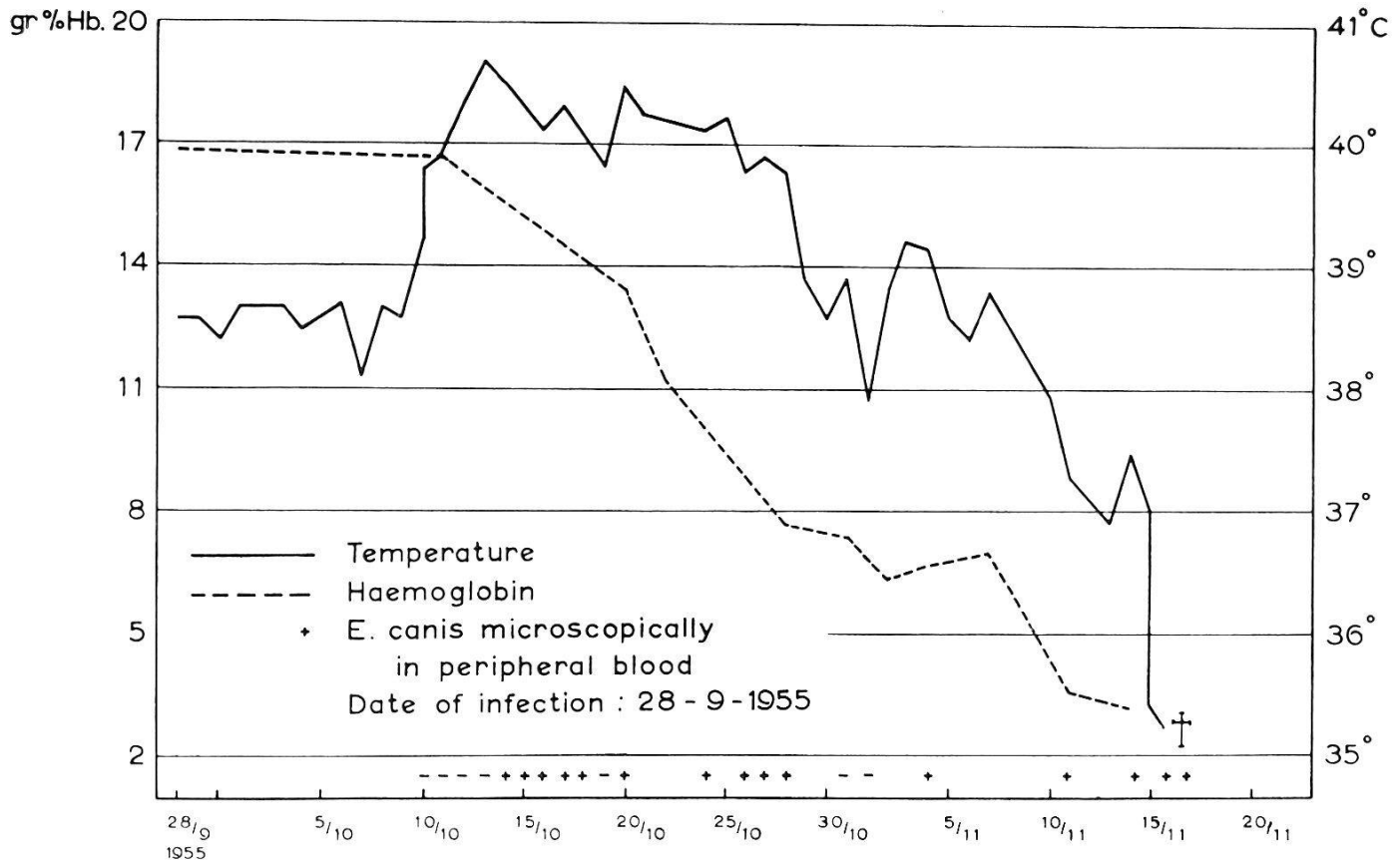


Fig. 1. Test-dog V, lethal infection with *Ehrlichia canis*.

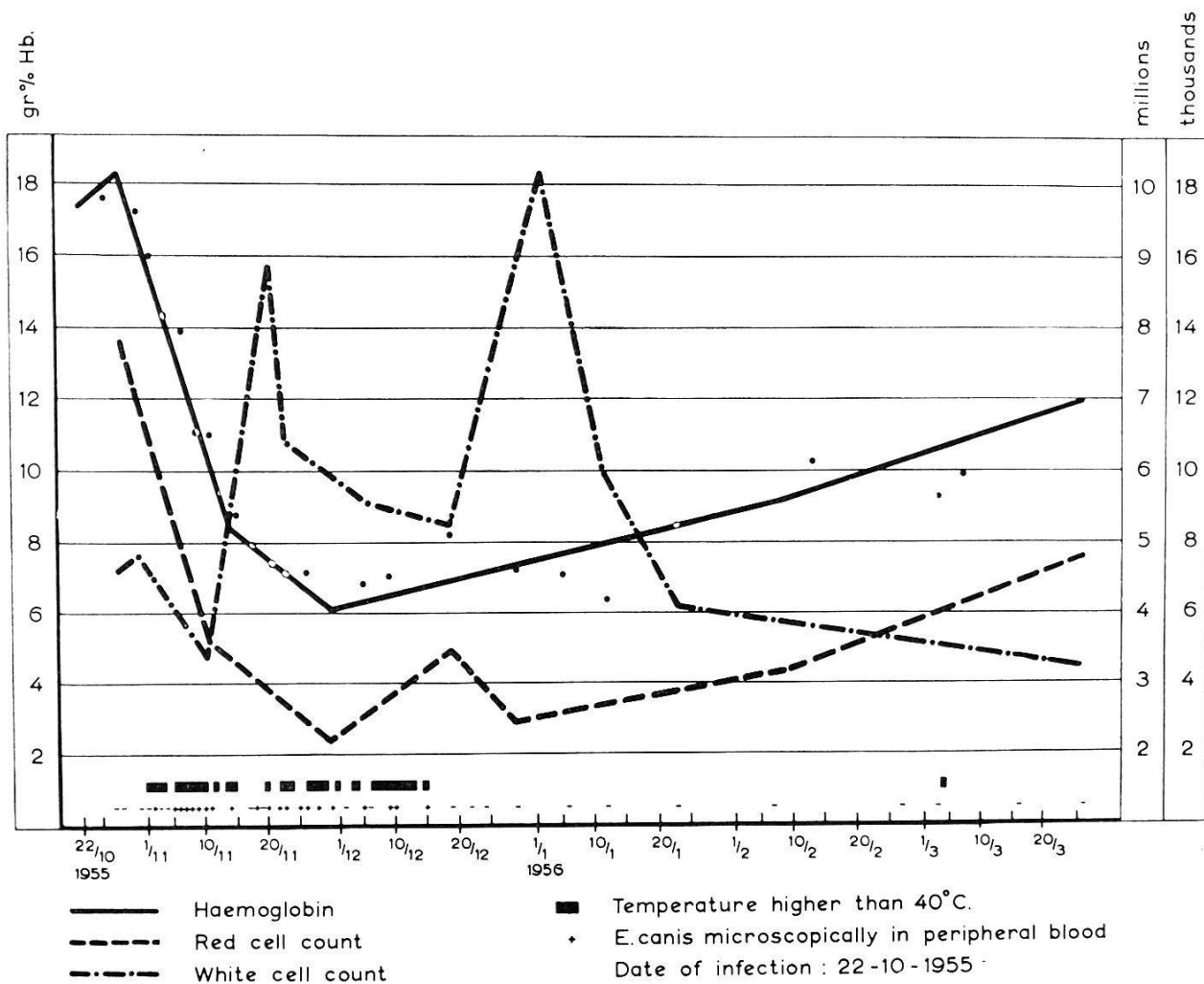


Fig. 2. Test-dog IV, infection with *Ehrlichia canis*, chronic course.

CHAEI and FIENNES (1942) also point out "atypical forms often resembling lymphocytes". Although in our preparations *E. canis* was indeed found in the lymphocytes (Fig. 3), this location is exceptional—just as is its presence in neutrophile granulated leucocytes (Fig. 4). Should, however, the interpretation of the leucocytes have given rise to the disagreement mentioned, then, in the extreme case, a lymphocytosis would be expected. However, no evidence of lymphocytosis was found.

In this connection the existence of a relative monocytosis in the first drop of blood from the ear may be pointed out; this was described for the first time in man by LUCEY (1921) and later confirmed by various research-workers (WOLLENBERG 1922). It appears that the percentage of monocytes in the first drop of blood from a human ear can be considerably higher than in the following drops. The same difference exists between the first drop of blood from a normal ear and from one that has been rubbed to produce an active hyperaemia; SCHÜFFNER and RUYS (1925) state that in this case the percentages may differ as much as 37. They explain this phenomenon as a result of the lowered speed of blood circulation in the capillaries of the ear, which causes the monocytes and, in a lesser degree, the lymphocytes to be concentrated in these blood vessels. In dogs we could not establish this relative monocytosis as a rule. It is possible that, owing to the mobility of the ear of the dog, the stasis of certain kinds of blood cells does not occur, or not to such a large degree, and that only under the influence of a disease the peripheral circulation is disturbed in such a way that the proportion of the white blood cells in the first drop of blood may no longer be considered representative.

The change in the white cell picture during the first 2 to 3 weeks of the disease is as follows: a shifting to the left, a slight reduction of the lymphocytes, a disappearance of the eosinophile granulated leucocytes and the occurrence of macrophages. Therefore no characteristic changes arise which might indicate an infection by *E. canis*.

The parasite:

DONATIEN and LESTOQUARD (1940) describe a developmental cycle in which the organism, during the first phase of the disease, occurs exclusively (LEVADITI et al. 1943) in the form of homogeneous bodies stained red with Giemsa—so called "corps initiaux"—situated in monocytes. A process of fragmentation results in an accumulation of granules which stain purple with Giemsa: "the morula". From these the third form will develop: the "corps

élémentaires”, which likewise stain purple, but which are loosely dispersed throughout the cell.

We have not been able to confirm the existence of these red-coloured “corps initiaux”. The first forms observed in the peripheral circulation 2 to 3 days after the commencement of the fever attack are “morulae”. Nor were “corps initiaux” found in the tissue-smears of dogs killed on the 5th and on the 9th day of fever. One red-coloured body resembling the description of DONATIEN and LESTOQUARD was found in a monocyte in the peripheral blood of a dog 20 weeks after infection and 6 weeks after splenectomy (Fig. 5). Other red-stained particles were indeed observed in the cell-plasma of leucocytes of *E. canis* infected dogs (Fig. 6); the microscopic picture as well as the fact that similar bodies were found in blood smears from a non-infected anaemic dog with leucocytosis, seems to indicate the presence of nuclear fragments ingested by phagocytosing cells.

The “morulae”, which sometimes are stained dark purple, may give the impression of being a homogeneous substance. However, after partial destaining with methyl-alcohol it can be seen that they consist of distinct, closely-packed particles (ROUSSELOT 1953).

We have therefore observed *E. canis* only in two forms, the “morula” and the “corps élémentaires” and are unable at this moment—as was COLES (1953) in his investigations on *R. conjunctivae*—to speak of a developmental cycle.

The theoretical possibility that the complete cycle occurs only after natural transmission can be excluded, as DONATIEN and LESTOQUARD have observed the “corps initiaux” also after experimental infection.

Culture and test animals:

In order to investigate the process of division of the organism an attempt was made to find a small test animal or a culture medium. It is known from the literature that besides dogs, monkeys (*Macacus inuus*) (DONATIEN and LESTOQUARD 1935) and jackals (*Thos mesomelas*) (NEITZ and THOMAS 1938) are also susceptible, although these animals do not show clinical symptoms. MALBRANT (1939) states that guinea-pigs react to an infection with fever, emaciation and signs of orchitis or vaginitis. He does not mention, however, whether *E. canis* was found in the guinea-pigs and whether transmission of tissue material from these animals to dogs has resulted in the disease. The results of MALBRANT's serological examinations would also indicate that his dogs had been infected with two different organisms, just as the

fact that he calls the rabbit "assez réceptif" towards *E. canis* (see Serology).

Several research workers (DONATIEN and LESTOQUARD 1936, ROUSSELOT 1953), on the contrary, mention negative results of the infection of guinea-pigs. Furthermore attempts have been made, without result, to infect mice, rabbits, sheep, cattle, monkeys (*Cercopithecus patas* and *Papio nigeriae*) and *Ictonyx striata* (family Mustelidae) (ROUSSELOT 1953).

Nor have our experiments with suckling mice, splenectomized mice and guinea-pigs been able to demonstrate a susceptibility after i.p. and i.c. infection with blood and tissue material originating from dogs with *E. canis*.

Referring to the description of CANHAM (1943) of organisms in monocytes of pigeons resembling rickettsiae, two pigeons were i.m. inoculated with blood from dogs. However, the *E. canis* could not be found again in the peripheral blood, nor did infection of dogs with the blood of the pigeons give any reaction.

A similar test with a cow had the same negative result.

In general it can be said that, with the exception of the dog, a susceptible test animal for laboratory use is as yet unknown.

We were not able to demonstrate a development of *E. canis* in fertilized hens' eggs incubated at 37°C. The eggs were inoculated in the yolk, in the allantois cavity and on the chorioallantois, respectively with blood and tissue (lung, liver, spleen) taken from dogs killed at the height of the disease. Six blind passages were made; without result 2 dogs were inoculated with egg material, one after the first and one after the last egg passage.

Therefore future research will have to investigate, among other things, the possibility of culturing *E. canis* in hens' eggs at a lower incubation temperature, in tissue culture, or in *Rhipicephalus sanguineus*.

Serology:

DONATIEN and LESTOQUARD (ROUSSELOT 1953) mention negative Weil-Felix reactions with serum from dogs infected with *E. canis*. ROUSSELOT (1953) likewise did not obtain agglutination with Proteus OX 19, OX 2 and OX K.

Besides reports of "weak positive" Weil-Felix reactions (CARMICHAEL and FIENNES 1942, ROUSSELOT 1953), mention must be made of the titer of 1:1250, found by LAWRENCE (1938) with Proteus OX 2 and serum of a naturally infected dog, whereas three control dogs reacted negatively.

MALBRANT (1939) describes agglutinations of Proteus OX 2 (1/500), Proteus OX K (1/100), Proteus OX 19 (1/200) and an

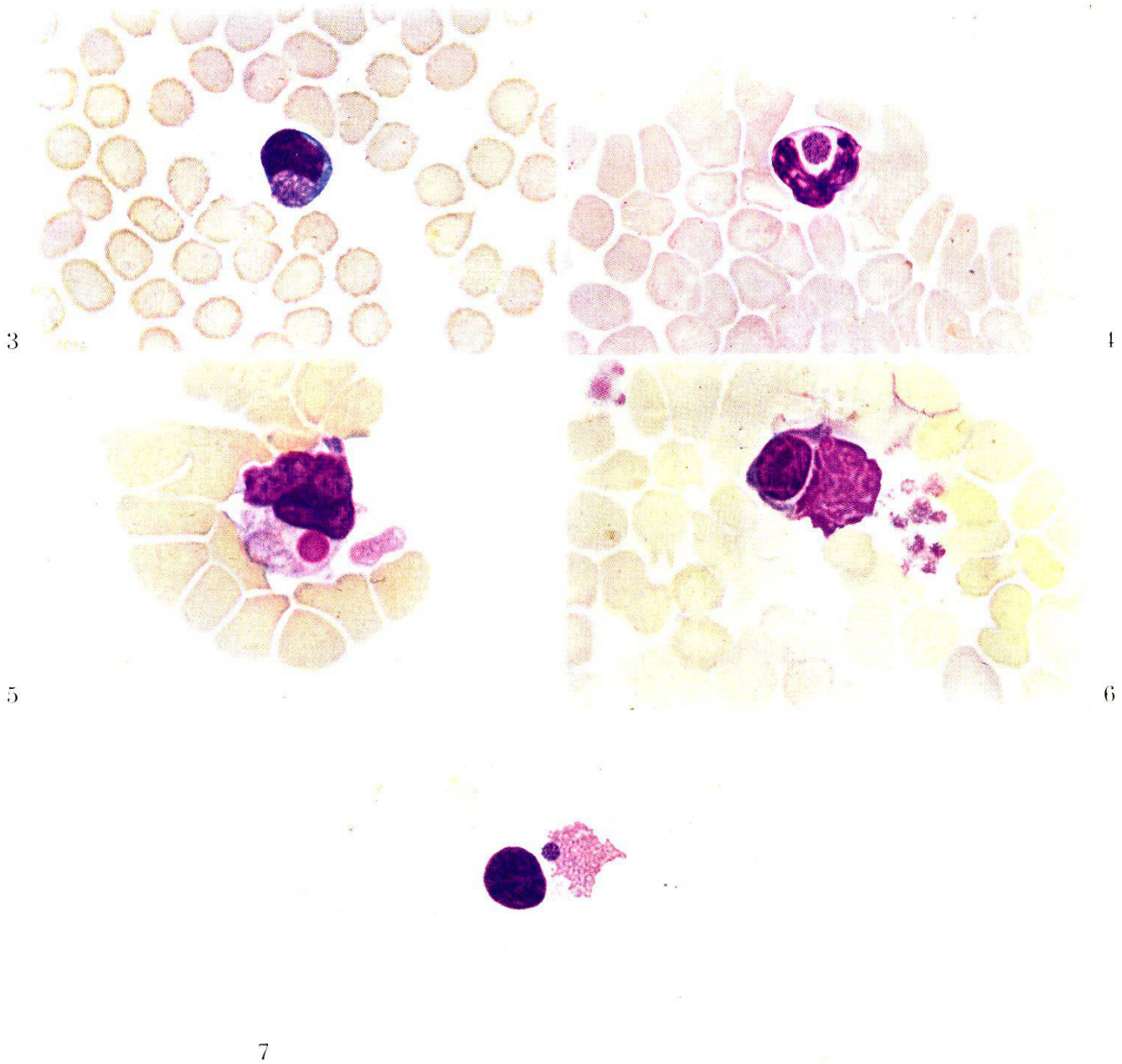


Fig. 3. "Morula" of *E. canis* in a lymphocyte.

Fig. 4. "Morula" of *E. canis* with red-stained matrix in a juvenile neutrophile leucocyte.

Fig. 5. "Corps initial?" of *E. canis* in a monocyte, see text.

Fig. 6. For explanation, see text.

Fig. 7. A "morula" and "corps élémentaires" in the peripheral blood.

All photographs $\times 920$.

agglutination (1/2000) of a *Proteus*-strain which was isolated from the blood of guinea-pigs injected with material from infected dogs. This last titer cannot be interpreted as a positive Weil-Felix reaction. In view of the above mentioned positive results of MALBRANT, concerning the infection of guinea-pigs, these serological reactions give rise to the supposition that his dogs, in addition to the presence of *E. canis*, have also been infected with one of the classical rickettsiae or with any other organism that shows a serological relationship to the *Proteus*-strains used. It might be possible to think of *R. conori*, since dogs may be infected latently with this rickettsia species; moreover, *Rhipicephalus sanguineus* serves as a transmitter for *E. canis* as well as *R. conori*. Several recent investigations have shown that a certain percentage of non-selected dogs possess antibodies which give an agglutination with *Proteus* strains. For example, SFORZA and SOLINAS (1949) found that 12 out of 159 dogs in Eritrea had a titer of 1:320. PLACIDI and SANTUCCI (1955) tested 80 dogs from the surroundings of Marseilles and obtained titers of 1:200 to 1:400 in 15 animals. HEISCH (1957) mentions that many dogs from Nairobi show a positive Weil-Felix reaction.

Our work upon the Weil-Felix reaction is too limited to attach great value to its negative result. Tests were made with *Proteus* OX 2, OX K, OX 19, and serum from 3 dogs, obtained 3, 5, and 8 weeks respectively after the infections with *E. canis*, but without showing agglutinations.

Dr. F. DEKKING (Institute of Hygiene, University of Amsterdam) was so kind as to examine samples of serum, taken at regular intervals in the complement fixation test with Q-fever antigen. The results were partly negative or could not be used because of anticomplementary qualities of the serum.

An effort to prepare an antigen for the C.F. test, from the lung of a dog killed in the acute stage of the disease had no satisfactory result.

In view of the data in the literature which do not exclude a positive Weil-Felix reaction during infections with *E. canis*, further serological research will investigate possible antigen relationships. But preference should naturally be given to more specific methods, such as rickettsia-agglutinations or C.F. test with various rickettsia antigens (PLACIDI and SANTUCCI 1954). Since it is improbable that a useful antigen can be obtained from the organs of dogs the development of an in vitro-culture will be the prerequisite for a specific serological reaction—just as is the case with *Neorickettsia helminthoeca* (Salmon "poisoning" disease) (PHILIP 1955).

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Résumé.

En pratiquant, avec *E. canis*, l'infection expérimentale d'animaux de laboratoire, des investigations ont pu être faites sur les questions suivantes : nomenclature, existence éventuelle d'un cycle, virulence, animaux d'essai, modification du sang, prémunition et sérologie.

Zusammenfassung.

Anlässlich experimenteller Infektionen von Versuchstieren mit *E. canis* bot sich Gelegenheit zur Untersuchung folgender Fragen: Nomenklatur, Vorhandensein eines Zyklus, Virulenz, Versuchstiere, Modifikation des Blutstatus, Prämunition und Serologie.
