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STUDIES ON BOVINE BESNOITIOSIS WITH SPECIAL REFERENCE TO THE AETIOLOGY

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STUDIES ON BOVINE BESNOITIOSIS WITH SPECIAL REFERENCE TO THE AETIOLOGY

I.—Introduction

Bovine besnoitiosis was recognised as a distinct disease in south-western Europe about 75 years ago. The discovery of the causative protozoon in the skin and subcutaneous tissues 38 years later in France opened several new fields of investigation. These included ascertaining the distribution of the disease, identifying the protozoan parasite, establishing the pathogenicity, determining the seriousness of besnoitiosis as an animal health and economic problem, looking for economically feasible methods of controlling the disease, attempting to determine the natural mode of transmission, and searching for chemotherapeutic agents for either curing or preventing the disease. Consideration of the results of the investigations, described below, makes it apparent that although advances have been made in the studies during the last five decades, the failure of establishing the natural mode of transmission and the absence of a specific remedy leaves bovine besnoitiosis as an important veterinary and economic problem.

In presenting the results of the investigations an attempt has been made not only to give a detailed account of the aetiology, but to include observations made on the pathogenicity, symptomatology, chemotherapy and immunology as they affect the biological properties of the infectious agent. For the sake of completeness, a brief review on the epizootology, diagnosis and prophylaxis will also be given. In doing so it will become clear what has been achieved and which avenues to future progress have to be followed.

Advances made in the studies on the aetiology during recent years have made it possible to dissociate the causal agent of bovine besnoitiosis from the eimerian parasites, the association of which, in the past, had lead to a hopeless classificatory and nomenclatorial confusion. It has at last become possible to give a clear definition of bovine besnoitiosis, and the two allied diseases occurring in equine and murine hosts.

Since the presence of bovine besnoitiosis was first recognised in the Rustenburg district nearly 20 years ago, an alarming spread to the adjoining districts has occurred in the Northern Transvaal, which constitutes one of the main ranching areas of the Union of South Africa. The economic implications of bovine besnoitiosis to stock owners are considerable. Although the direct losses do not exceed ten per cent, the indirect losses as a result of poor productivity due to the very long convalescent period, the temporary or permanent sterility of affected bulls, and the heavily parasitized hides, rendered useless for the leather industry, are extremely high.

II.—Definition

Bovine besnoitiosis is an acute, subacute or mild protozoan disease caused by *Besnoitia besnoiti* (Marotel, 1912). It is characterized by pyrexia, anorexia, malaise, swelling of the palpable lymphatic glands, scleritis, lachrymation, anasarca, scleroderma titis, alopecia, rhinitis, pharyngolaryngitis, tracheitis and orchitis. Cattle that survive either an acute or subacute infection retain a chronic sclerodermatitis, rhinitis, pharyngolaryngitis, tracheitis and scleritis, accompanied by a permanent or temporary sterility in bulls, and a premunity which persists for life in both sexes.

III.—Synonyms

Bovine cutaneous globidiosis, Bovine cutaneous sarcosporidiosis, Elephant skin disease of cattle; Huidsarkosporidiose van beeste, Huidglobidiose van beeste, Besnoitiose van beeste, Olifantsvelsiekte, Dikvelsiekte (Afrikaans); Huidglobidiose van runderen, Besnoitiose van runderen (Netherlands); Haut Sarcosporidiose der Rinder, Haut Globidiose der Rinder, Besnoitiose der Rinder (German); Anasarque du boeuf, Elephantiasis du boeuf, Sarcosporidiose cutanée chez bovins, Globidiose cutanée du boeuf, Besnoitiose du boeuf (French); Anasarca bovina, Sarcosporidiose cutanea bovina, Globidiose bovina por *Globidium besnoiti*, Besnoitiose bovina por *Besnoitia besnoiti* (Portuguese); Dillag (Arabian).

IV.—History

The history of bovine, equine and murine besnoitiosis is interesting. Perusal of the literature shows that observations conducted on domestic animals in Europe and Africa were accompanied by a remarkable confusion about the identity of the causal agents, the nomenclatorial history of which will be discussed below (*vide infra*—Aetiology, Classification).

During the last five decades the disease in cattle was known as either bovine cutaneous sarcosporidiosis (Besnoit & Robin, 1912, 1913 a, b, 1914; Cuillé, Chelle & Berlureau, 1936) or bovine (cutaneous) globidiosis (Cuillé & Chelle, 1937; Hofmeyer, 1945; Leitao, 1949; Pols, 1954 a, b; Henning, 1956), and that in horses as equine (cutaneous) globidiosis (Bennett, 1933; Schulz & Thorburn, 1955; Henning, 1956). Recently, however, it became apparent that the correct generic names are bovine and equine besnoitiosis.

A disease referred to as " l'elephantiasis et de l'anasarque du boeuf " was described by Cadéac (1884) in cattle from Southern France. Subsequent investigations by Besnoit & Robin (1912) revealed the presence of an extremely large number of thick-walled spherical cysts, harbouring numerous spores, in the skin and subcutaneous tissues of affected cattle from the Pyrenees. They tentatively referred to the causal agent as a *Sarcocystis* sp. Marotel (1912) pointed out that nothing similar had been found previously in cattle, and that the names of the investigators deserve to be connected with this wonderful discovery. He, therefore, proposed the species name *besnoiti*, and used the combination *Sarcocystis besnoiti* for the causal agent.

Franco & Borges (1916) gave a detailed account of the disease and stated that, since 1885, it has been encountered frequently in slaughter cattle at the abattoir in Lisbon, particularly in animals derived from the province Alentejo in Portugal. In their account they reported that not only was the skin affected, but that the aponeuroses revealed a massive infection of cysts, giving the impression that these tissues had been sprinkled heavily with sand. Infected carcasses were declared unfit for human consumption. Since then the disease has been encountered in the Sudan (Bennett, 1928), and in several other African states listed in the subjoined Table 1. (*vide infra*—Distribution).

The first record of equine besnoitiosis is that of Henry & Masson (1922), who encountered the infection in a horse in the vicinity of Rheims in Northern France. Several years later Going (1927) and Bennett (1927) encountered this disease in horses from the Sudan. It was later established that the horses originated from the Nuba Mountains situated in the southern part of the province Kordofan. The economic importance of this infection was brought to light by a series of official

enquiries carried out by Capt. H. B. Williams, in whose opinion the horse-owning tribes were well acquainted with this disease. He reported that the Hawasma Arabs of Rashad Merkuz, not many years ago, possessed 600 to 800 horses, of which less than 50 remained at the time of enquiry. This loss was attributed to a disease which the Arabs named " Dillag ", apparently equine besnoitiosis.

Although Babudieri (1932) and Enigk (1934) stressed that the cutaneous parasites of cattle and horses are not related to the intestinal parasites (*Globidium* spp.) of these animals, many investigators persisted in referring to these diseases caused by the former organisms as cutaneous globidiosis.

The turning point in the investigations on besnoitiosis was the discovery by Jellison (1953), who found pearly white cysts, harbouring numerous spores, in the mediastinum of a white-footed mouse [*Peromyscus maniculatus artemisae* (Wagner)] However, it was left to Frenkel (1953 a) to show that this parasite, which multiplies by binary fission, is not only related to *Besnoitia besnoiti* but also to *Toxaplasma gondii.* These studies were followed by those of Pols (1954 a, b) who found that *B. besnoiti* also reproduces by binary fission, and that it is morphologically indistinguishable from *T. gondii.*

V.—Distribution

The distribution of bovine, equine and murine besnoitiosis, together with the names of investigators responsible for determining their presence in different countries, are listed in Table 1.

From the description given by European investigators it becomes apparent that bovine besnoitiosis occurs enzootically in France and Portugal. Leitao (1949) reported that cases of this infection were frequent in cattle imported into Portugal from Angola. Whether or not these animals served as additional reservoirs of *B. besnoiti* for the infection of indigenous Portuguese cattle could not be determined.

Since the recognition of bovine besnoitiosis in South Africa in 1941 (Hofmeyer, 1945), surveys by Thomas *et al.* (1943) revealed that this disease is widely distributed in the Rustenburg district. Farmers in this area stated that they had seen this disease for the last decade but that they had mistaken it for bovine mange. Subsequent investigations by veterinarians, listed in the appended Table 1, have disclosed that the disease is gradually spreading, and at present it is widely distributed in the north-western, northern and north-eastern bushveld area of Transvaal. In addition, it has been established that the infection also occurs in South West Africa, Bechuanaland, Angola and the Belgian Congo.

It will be noticed from the available records (Table 1) that only a single case of equine besnoitiosis is recorded from Europe, that it appeared as an important malady in Sudanese horses, that it occurs sporadically in the Union of South Africa with its large equine population, and that it seems to be absent from the remaining African territories, in which bovine besnoitiosis occurs enzootically. Its apparent absence may be attributed to the incidence of horsesickness in Angola, Bechuanaland, the Belgian Congo and Ovamboland, as reflected by the extremely low equine population.

Up to the present, murine besnoitiosis has only been recorded in a white-footed mouse from the United States of America.

TABLE 1

Continent	Country	Animal	Reference
Europe.....	Southern France.....	Cattle	Cadéac, 1884; Besnoit & Robin, 1912, 1913, 1914; Cuillé, Chelle & Berlureau, 1936; Cuillé & Chelle, 1937; Barrairon, 1938.
	Northern France (Rheims)....	Horse	Henry & Masson, 1922.
	Portugal (Alentejo).....	Cattle	Franco & Borges, 1916.
	Portugal (Imported) cattle from Angola)	Cattle	Leitao, 1949.
Africa.....	Angola (Alvito, Beja, Elvas, Evora, Grandola, Moura, etc.)	Cattle	Leitao, 1949.
	Bechuanaland (Serowe).....	Cattle	Director of Veterinary Services, 1957.
	Belgian Congo (Ruanda-Urundi)	Cattle	Colback, 1951; Herin, 1952.
	South West Africa (Ovamboland)	Cattle	De Boom, 1947.
	Sudan.....	Horses	Bennett, 1927–1939; Going, 1927.
	Sudan (Nuba Mountains in Southern Kordofan)	Horses	Williams, 1932.
	Sudan.....	Cattle	Bennett, 1928.
	Union of South Africa—Transvaal districts:—		
	Lydenburg.....	Cattle	Meeser, 1954.
	Marico.....	Cattle	Thomas, 1950; Schulz, 1951.
	Pietersburg.....	Cattle	Van der Merwe, 1953.
	Potgietersrus.....	Cattle	Hurter, 1950.
	Pretoria.....	Cattle	Van der Vyver, 1945, De Wet, 1950; Pols, 1953.
	Rustenburg.....	Cattle	Hofmeyr, 1945; Thomas, Frean & Neitz, 1943; Louw, 1954.
		Horse	Thomas, 1950.
	Soutpansberg.....	Cattle	Thomas, 1950; Mansvelt, 1953.
	Waterberg.....	Cattle	Thomas, 1943.
		Horse	Thomas & Adelaar, 1947.
	Eastern Cape Province District—Grahamstown.....	Horse	Schulz & Thorburn, 1941, 1955.
	Natal Districts—		
	Umfolozi (Empangeni)....	Horses	De Kock, 1947.
	Vryheid.....	Horses	Lamprecht, 1950.
United States of America	Hamilton in Montana.....	White-footed mouse	Frenkel, 1953 b.

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VI.—Aetiology

Besnoitia besnoiti (Marotel, 1912).

Synonyms: *Sarcocystis besnoiti* Marotel, 1912.
Gastrocystis besnoiti (Marotel, 1912).
Globidium besnoiti (Marotel, 1912).

(*a*) *Morphology*

Fairly comprehensive accounts of the histological studies on the causal agent of bovine besnoitiosis, accompanied by a series of microphotographs, have been given by Besnoit & Robin (1912) and Franco & Borges (1916). Although the descriptions were limited to a study of advanced stages of the development of the organism, they nevertheless served as a basis for later investigations on *B. bennetti* Babudieri, 1931, by Bennett (1933), *B. jellisoni* Frenkel, 1953, by Frenkel (1953 a, 1954) and *B. besnoiti* (Marotel, 1912) by Pols (1954 a, b).

It will be seen from the description, given below, that the advances made in the research resulted from the discovery of laboratory animals susceptible to the *Besnoitia* spp., and the availability of improved staining techniques. It has thus become possible not only to supplement the original observations, but also to modify certain views held by previous investigators. The progress made comprises the determination of the vertebrate life-cycle of the infectious agent, and the origin and development of the cystic wall, as well as the identification of certain chemical components of the parasite and cystic wall, as revealed by the staining reactions.

The present account of the morphology of *B. besnoiti* is based chiefly on the observations made on rabbits used for the serial passage of this parasite. Recovery that followed the artificial infection made it possible to trace the development and behaviour of the parasite in the circulating blood and tissues, and to observe the response of the host to the infectious agent during the period of the febrile reaction and the ensuing chronic phase of the disease. Observations recorded by Pols (1954 a, b) will be recapitulated and extended to include the studies on the origin and development of the cystic wall which, up to the present, had not been fully understood. Observations made on rabbits will be compared with those made on cattle and a single goat that developed a typical but non-fatal besnoitiosis reaction.

The forms of the organism, circulating in the bloodstream and seen in the blood, lung and testis smears of rabbits, vary in size from 5 to 9 microns in length and from 2 to 5 microns in width. Similarly, there is a variation in shape. Oval forms slightly pointed at one end are the most common in blood smears. Curved forms with rounded ends (banana-shaped) and crescentic types are found more rarely, except in lung and testis smears where they are quite common. They are also more frequent in smears obtained late during the temperature reaction, when the number of monocytes is decreasing. They give the impression of being more shrunken or mechanically compressed. Only a few round forms were observed

With Gram stain, the trophozoites give a negative reaction, and stand out clearly when stained with Moeller's method: a distinct blue on a reddish background, but internal structural detail is by no means distinct. With Giemsa stain, the cytoplasm is granular and stains blue, usually slightly lighter at the one (blunt) end. At this end one often discerns a yellowish red " caplike " area from which fine striations radiate toward the nucleus. Occasionally darker blue granules, as well

as vacuoles, are noticed. They are distributed throughout the cytoplasm, but more towards the pointed end. With Nile blue sulphate and Periodic-Acid-Schiff reagent, they react positively; pretreatment with saliva abolishes the latter reaction. It is, therefore, concluded that the cytoplasm contains glycogen. (Interestingly enough decolorisation of previously P.A.S. stained preparations, and subsequent treatment with saliva do not abolish the P.A.S. reaction). Other staining reactions were performed on sections of skin. The results will be mentioned when dealing with the cyst forms. The banana- and sickle-shaped forms, by nature of their denser cytoplasmic structure, stain more intensely and detail is less distinct.

The nucleus is situated near the centre. It may, however, occur in various positions and does not conform to a definite pattern or size. In the oval-shaped parasite it varies from a fairly compact form to a loose network of chromatin. With Giemsa it stains the usual reddish purple, darker when the nucleus is more compact. The latter appearance is the one seen in the crescent-shaped organisms as a rule. The oval form may be binucleate. On application of the Feulgen technique, the nuclei take on a very faint tinge of purple, in sharp contrast to the brilliant reaction given by the nuclei of the leucocytes.

The intracellular parasites do not differ from the extracellular ones, except that in blood smears the banana- and sickle-shaped forms are often not as frequent. As can be expected, extracellular parasites are difficult to discern in sections, and the intracellular ones appear much smaller and more crescent-shaped. Great numbers of divisional forms are seen in different stages of development, both intra- and extracellularly. The most common of these is a binucleate one, where division of the cytoplasm is in the process of taking place or has just been completed. Otherwise two parasites may be seen closely approximated as to suggest early separation. Multiple divisions do occur; such are exemplified by cytoplasmic masses containing four to eight nuclei more or less regularly spaced.

In skin biopsies and organ specimens taken at autopsy, sectioned singly and serially and stained with haemalum-eosin, organisms, after much searching, could be seen lying both singly and in groups between the connective tissue fibres. It is impossible to state whether they were in the lymphatics or not. Infrequently organisms are also seen in wandering or fixed histiocytes, either singly, in pairs or in larger groups in a vacuole in the host cell. As stated above, such organisms in section appear in a much contracted form; they measure approximately 3 microns in length and 1·5 microns in width. Depending upon the number of parasites in them, vacuoles may be quite small, measuring approximately 8 microns in diameter during the third week after infection of the subject. In specimens taken at progressively longer intervals after infection, they become much larger; eventually, at about nine weeks, they range from 80 to 120 microns in size with extremes of 50 to 200 microns. They then contain a correspondingly increased number of organisms. These are laden with glycogen as may be inferred from the brilliant P.A.S. reaction which is abolished after treatment with saliva, leaving only a cytoplasmic hull. With Hale's stain the cytoplasmic contents react almost equally well, indicating the presence of acid mucopolysaccharide. The latter tends to be concentrated towards the blunt end. However, the quantity present in any one individual organism varies from virtually nothing to relatively large accumulations, in contrast to the glycogen which is virtually always present. The constant positive reaction to silver impregnation, performed according to the Gömöri technique, could also be ascribed to this fact. As in the case of the blood parasites, the nuclei react very faintly indeed to Feulgen's staining method. Only by employing Wratten's filters could any satisfaction be obtained that there really was a purplish colour.

When one or a few organisms occupy the vacuole there is a clear space around the parasites. When present in larger numbers, they are more tightly packed, leaving little or no intervening clear areas. In large vacuoles the outer edge forms a distinct membrane which has been referred to as the inner membrane (Pols, 1954 b).

The infected host cell is seen to contain two or more nuclei. These are very vesicular with a delicate nuclear membrane and numerous enlarged nucleoli. In distinct contrast to the nuclei of the organism, they are clearly Feulgen positive, although their vesicular nature makes for a scant distribution of positive material distributed just under the nuclear membrane and here and there towards the centre, as discrete rounded knots. The nucleoli are Feulgen negative, with occasionally a narrow sickle-shaped positive edge. Because of the vesicular nature of these nuclei, their staining reaction is somewhat dulled when seen against the green background of underlying Feulgen negative cytoplasm. The nuclei increase vastly in number and generally form a radiating crown at some distance from the periphery of the cell. Mitoses are observed infrequently. When division occurs, all nuclei present in one cell divide simultaneously. Ultimately, due to parasitic encroachment, the nuclei become wrinkled, the nuclear membrane folded and the whole nucleus elongated. Finally they become pycnotic and compressed to a very narrow elongated strip of nuclear material. The cytoplasm of the host cell enlarges to gigantic proportions, becomes deeply basophilic and markedly granular.

Between the nuclei and the periphery of the cell it is somewhat lighter, and here large, distinct, perfectly clear vacuoles are seen in embedded sections. Some of these vacuoles may actually be interpolated between the nuclei. In this area too, vacuoles containing parasites occur. The centre of the host cell shows an enormously enlarged spherical centre which is much less basophilic. In it the very extensive negative image of a Golgi apparatus may frequently be seen. The outline of this central area is often accentuated by a pallisade of dark, basophilic, cytoplasmic streaks lying around it. They may be shown up well in iron haematoxylin preparations.

In the earlier phases of the host cell's development, its cytoplasm gives only a light P.A.S. positive reaction. When it has attained its maximum development large granules of intensely red staining material are scattered throughout the central zone, tightly packed in large masses immediately around it and between the nuclei, whereas the peripheral area is relatively clear. The reaction is abolished by pretreatment with saliva. As the parasite enlarges the P.A.S. reaction is lost, although the rate of loss does not appear to be constant. At the cell membrane there is a distinct P.A.S. positive zone, which soon merges with the rest of the capsule; its exact situation, whether intra- or extracellular, is very difficult to determine. The latter interpretation is probably correct. The positive reaction remains despite salivary digestion. Despite the strong P.A.S. reaction exhibited by the cytoplasm of the host cell during its phase of maximal development, it was never found to react to Hale's stain for acid mucopolysaccharide. The central zone of the host cell's cytoplasm is also argyrophilic. The same granular appearances as described for the P.A.S. reaction is seen after Gömöri's reticulum impregnation. Later the argyrophilic granules condense at the periphery of the central zone. With development of the parasite, the argyrophilic granules are pushed aside in irregular masses and eventually the argyrophilia disappears.

With enlargement of the parasite containing vacuoles, the regularity of the host cell structure is disturbed, as already will have been inferred from the foregoing description of the various staining reactions. When packed with organisms, the

cytoplasm is compressed to a very narrow rim (" the intermediate membrane "), and between this and the inner membrane a varying number of compressed host cell nuclei may be seen. Sometimes the occurrence of organisms in different vacuoles in one host cell produce a bi- or tri-locular appearance. No nuclei appear in the septa.

During the development and regression of the host cell, the following sequence of events is observed around it. At first it becomes surrounded by a homogeneous matrix, around which young collagenous fibres are laid down. The matrix stains poorly, gives a negative reaction with P.A.S. and van Gieson, and stains a light blue with Hale's stain, In it delicate fibres, argyrophylic, fuchsinophilic, P.A.S. and Hale positives are seen to course. It is probably a condensation of these fibres which is responsible for the narrow rim of P.A.S. and Hale positive, argyrophilic material demarcating the boundary between the future capsule and the host cell, and already referred to when dealing with the P.A.S. reaction of the latter's cytoplasma. It must be mentioned, however, that the argyrophilia was not a constant feature.

With enlargement of the host cell the capsular zone becomes progressively more and more positive to the P.A.S. reaction, and at the same time stains intensely with Hale's stain. With the latter stain, a thick, deep blue capsule, with a very narrow, lighter staining inner rim then surrounds the host cell. As the P.A.S. reaction gains in intensity so does the capsule become more and more fuchsinophilic. At the same time, the intensity of the Hale reaction decreases, and the whole process goes hand in hand with the deposition of collagenous fibres. In developing cysts, these fibres can be traced, distinctly running from the surrounding connective tissue. Fibroblasts accumulate more or less regularly around this collagenous mass. Eventually the collagenous fibres become hyalinised, intensely fuchsinophilic and P.A.S. positive. Especially with the latter reaction the inner part of the cyst wall is intensely coloured. Whereas in the earliest stages of cyst development salivary predigestion affects the subsequent P.A.S. reaction but slightly, it produces quite a pronounced effect in the fully developed cyst. Despite this, a considerable amount of P.A.S. positive material remains, especially towards the inner part of the cyst wall. In this stage Hale's stain produces only narrow, complete or incomplete ringshaped zones, or granular accumulations of blue staining material. Where capsules abut on each other, similar material is found in considerable quantity in the intervening spaces. Argyrophilic fibres become less pronounced as the cyst approaches maturity

In cattle, *B. besnoiti* presents essentially similar morphological features. The circulating organisms, of which only very few were seen, did not differ in any way. The fully formed cyst in cattle is much larger than that found in the rabbit, measuring up to 600 microns in diameter. When seen singly they are spherical or sometimes slightly spheroidal. When present in large numbers they may be compressed; the outer membrane or cyst wall then becomes confluent. Organisms expressed from the cysts and examined in smears are mostly crescentic and measure on an average 2 by 7 microns. In saline suspension they exhibit moderate motility by flexing and slightly contracting and then extending. The parasite seen in the goat did not differ from that seen in cattle. Around fully formed cysts no further tissue reaction is elicited. In infections of long standing they appear as perfectly bland parasites with little indication of their genesis. In cattle, an occasional degenerating cyst was observed with remnants of the now incomplete capsule and the central necrotic and calcifying debris, with a localised tissue reaction indicating resorption.

(*b*) *Multiplication*

B. besnoiti, like *B. jellisoni*, multiplies within the host cells by repeated binary fission (Frenkel, 1953 a; Pols, 1954 a, b). Multiple divisions do occur, but when compared with the former, they are so rarely observed as to lead one to suspect that they are aberrant forms (Pols, *loc. cit.*). Reproduction continues within monocytes, and possibly also in neutrophiles, until they rupture and is then again resumed after parasitic invasion of new host cells. In the hypertrophied histiocytes, multiplication persists until the resulting cyst, with its firm capsule, is filled to capacity. In this situation parasites remain dormant for periods of up to ten years, after a natural infection (*vide infra*—Immunity). Absence of any degenerative changes of cysts during this long period, as determined histologically, suggests that the parasites survive in chronically affected cattle for even longer periods.

(*c*) *Habitat*

Bennett (1928) described the presence of *Besnoitia* parasites, initially termed " sarcospores " and later, after Wenyon's identification, " Globidium spores ", in large numbers in blood smears obtained by skin punctures from affected horses. On taking blood from the jugular vein for the preparation of smears, parasites could not be found. He concluded that probably accidental contamination from incised cutaneous cysts had occurred. In view of the fact that blood is only infective during the primary stage of the disease (Cuillé, Chelle & Berlureau, 1936; Pols, 1954 a), and Bennett's cases seemed to be advanced, his final conclusion is most likely to be correct. Cuillé & Chelle (1937) spoke of a " virus " in the blood of cattle during the primary stage of the disease, and tried to determine whether this infectious agent was attached to the erythrocytes or free in the plasma, but without success. It may thus be presumed that *B. besnoiti* had not been observed previously in cattle in any form other than that in cysts.

In order to determine the habitat of the parasite seven head of cattle, listed in Table A of the Appendix, were subjected to daily blood and lymphnode smear examinations. In four of these cases extracellular parasites were seen in blood and lymphnode smears, on one day only during, or, at the most, two days after the height of the febrile reaction. In one animal they appeared in the blood and lymphnode smears, in two cases in blood smears, and in one in a lymphnode smear only. One or, at the most, three parasites could be found per smear. Parasites in the circulating blood and lymphatic tissues of cattle are thus extremely rare. The appearance of parasites in cysts situated in the skin and adjacent connective tissue has already been commented upon (*vide supra*—Morphology).

In contradistinction to cattle, the rabbit forms a far better subject for the study on the habitat of the parasite. In 80 per cent of the 440 rabbits, used for the 125 serial passages, parasites could be demonstrated in blood smears. It may be interesting to note that from generation 119 to 125 the percentage of positive smears dropped to about 60 per cent.

In rabbits parasites were demonstrable in the circulating blood for either one or for six consecutive days, from about the peak of the thermal reaction or shortly thereafter. The average period was about two days. It is possible that in some cases the microscopic infection may have been longer, since rabbits with the best reactions were selected, as a rule, as donors and were killed for the collection of blood.

For the demonstration of parasites, smears were prepared by pushing the upper slide, and drawing the drop of blood behind it. In this way the blood formed a flare at the end of which the majority of leucocytes collected. An increase in monocytes, best seen here, presaged the presence of parasites within the next few days. This site, too, is the most favourable for finding parasites. In the average positive smears one can expect one trophozoite per four or five microscopic fields. As a rule the distribution is irregular. The majority of parasites in blood smears were extracellular. Intracellular parasites in monocytes occurred less frequently. Organisms were encountered occasionally in neutrophiles.

After death, smears were made from the lung, liver, spleen and testis from a relatively large number of rabbits, and also in some cases from the brain, kidney, lymphnodes, subcutaneous oedematous fluid and faeces. Provided the period intervening between the height of the thermal reaction, and organ smear examination did not exceed ten to 12 days, parasites could be demonstrated in lung and testis smears, rarely in those of the liver and spleen, while all the remaining preparations proved to be negative. On some occasions lung and/or testis smears were positive when no parasites could be found in blood smears.

Although fully aware of the naturally occurring polymorphism of *Besnoitia* parasites, and of possible artefacts arising during the preparation of smears, the writer considers the increased number of so-called contracted forms, i.e. banana- and sickle-shaped types, during the later stages of the febrile phase of the disease, as significant. They are those forms which are subsequently seen in the interstices of cutaneous and subcutaneous connective tissue. Here they enter the tissue histiocytes which either have undergone reactive changes just prior to penetration or react soon thereafter. The reaction of the host cell is evidenced by multiplication of the host cell nucleus, enlargement of its cytoplasmic mass, i.e. the formation of a plasmodium, and the appearance of a distinct clear vacuole in which the parasite is seen to lie. More than one of such parasitized vacuoles may develop. The parasite proliferates by binary fission and, at the same time, the vacuole enlarges. Meanwhile, cytoplasmic enlargement and nuclear multiplication of the host cell continue until its body attains gigantic proportions. Large, oval, vesicular nuclei with prominent nucleoli are disposed, shingle fashion, around the periphery in a zone of more intensely basophilic cytoplasm. Towards a centrally situated, spherical, well demarcated zone, this basophilic material is concentrated in frequently distinct, radially disposed, rod-like structures. The large central zone, which stains lighter, is interpreted as an hypertrophied centrosome in which canaliculi of an equally enlarged Golgi apparatus can be distinguished as a negative image in ordinary preparations. The enlarging vacuoles with parasites, at this stage, still occupy a segment or segments of the peripheral zone in between the host cell nuclei. At this stage the parasites are still surrounded by a clear rim. During subsequent enlargement they come to fill the vacuole completely and lie tightly packed. As a result, the host cell's cytoplasm and nuclei become crowded towards one end. When two or more vacuoles are present in the same cell their opposed sides are compressed into a thin septum containing no nuclei, although the latter may extend towards the septum where the opposed edges of the vacuole diverge. Eventually cytoplasm and nuclei are reduced to a very narrow, dark staining rim, the former only visible in fortunate sections of the cyst. During this process young connective tissue fibres are laid down around the host cell by fibroblasts in the vicinity. These fibrils collagenize, and eventually hyalinize to form the well known, thick, more or less homogeneous cyst wall.

The histo-chemical reactions performed indicate that there is a temporary development of polysaccharide material in the host cell during the earlier phase of parasitism, especially in the interzonal region near the periphery of the greatly enlarged centrosome. According to the Hale reaction this is not acid mucopolysaccharide. The vesiculation and chromatin margination of the host cell nuclei is clearly revealed by the Feulgen reaction.

The pericellular connective tissue reaction yields a histochemical response which, in all respects, is similar to that of fibrous connective tissue formation. Young argyrophilic fibrils condense against the cyst wall. They are strongly positive for acid mucopolysaccharides. In the surrounding matrix further fibrils are laid down, strongly positive for the same substance. Further laying down of fibrils gives the forming cyst wall a concentric effect. A process of collagenization then sets in, the argyrophilia and reaction for acid mucopolysaccharides decrease but, at the same time, the mucopolysaccharides and glycogen content increases, the accumulation being greater in the older, i.e. inner part of the cyst wall. Eventually this part remains strongly P.A.S. positive, even after diastase treatment, indicating probably convergence of glycogen into mucopolysaccharide.

It is thus quite clear that the cyst in *B. besnoiti* is host-formed. There is no reason whatsoever to accept a parasitic origin of the cyst wall merely on grounds of its argyrophilia or of its P.A.S. positive reaction. At most, the residual mucopolysaccharide content, concentrated along the inner part of the cyst wall, indicates a reaction to the presence of the parasite. The clearly demonstrable fibrils of connective tissue converging around the parasitized cell offer definite histological proof. Also it is inconceivable that parasitic material could produce a cyst wall through the intervening medium of the host cell cytoplasm.

According to Frenkel & Friedlander (1951) Weinman was probably the first to use " pseudocyst " as a noncommital descriptive term, since there is no proof that the cyst wall was elaborated by the organism. Frenkel & Friedlander (*loc. cit.*), to whom it appeared that the pseudocystwall of *Toxoplasma* was likely to be derived from the micro-organism, employed the term cyst or pseudocyst interchangeably. In order not to confuse matters by an introduction of new terms, the author proposes that the term pseudocyst be restricted to those cyst-like structures formed by the host under the influence of the parasite. In this sense Jacobs (1956) employed the term with reference to *Toxoplasma*, although he contrasted the latter to *Besnoitia* and *Sarcocystis*.

The above description can be briefly summarized as follows: After the artificial infection of a susceptible host and the lapse of a due incubation period, a hyperthermia, persisting for several days, is observed. During the period of the febrile reaction parasites appear in the blood stream in small numbers in the case of cattle, and in relatively large numbers in rabbits. Simultaneously they are demonstrable in the superficial lymphnodes of cattle and in the subcutaneous oedematous fluid, lung, testis, liver and spleen of rabbits, either by means of microscopic smear examination or subinoculation tests. Based on the relative numbers of organisms found, observations indicate that they appear primarily in the blood circulation and probably in the lymph stream. Here they occur extracellularly in more or less oval forms, but more acutely rounded at one end. It is considered that these forms invade the greatly increased numbers of monocytes, where they undergo multiplication, eventually to be set free into the circulation again. It must be stressed that intracellular proliferating forms lie in the cytoplasm of the host cell unencapsulated by any structure. They are not seen inside a distinct vacuole,

A number of forms, in which the cytoplasm is in various stages of longitudinal cleavage subsequent to nuclear division, are seen extracellularly in air-dried smears. Despite the care with which these smears are prepared, the relative scarcity of such forms leads one to consider them as divisional stages mechanically freed as a result of cell rupture consequent upon drawing the smear. It is not possible to state whether or not the host cell undergoes degeneration and dissolution in the process of parasite liberation.

After cessation of the fever the parasites disappear from the blood circulation and reappear in actively growing histiocytes situated in the fibrous tissue of the cutis, subcutis, aponeuroses, testis, sclera and mucous membrane of the upper respiratory tract. In the latter two sites numerous cysts can be detected readily with the naked eye. The host cell becomes markedly hypertrophied, and shows a variable number of nuclei which multiply by binary fission. The parasites create a vacuole, reproduce rapidly and push the nuclei to the periphery of the host cell. Cysts reach maturity approximately six to eight weeks after an artificial infection.

(*d*) *Life-cycle*

Within the vertebrate host *B. besnoiti* shows an asexual reproduction in monocytes, histiocytes and possibly also in neutrophiles. The natural mode of transmission of besnoitiosis is unknown. In the event that it should be found that an arthropod serves as a biological transmitter, it is possible that a sexual phase in the invertebrate life-cycle may be disclosed.

(*e*) *Cultivation*

No attempts have been made to grow *B. besnoiti* in chick embryos and tissue cultures.

(*f*) *Resistance to physical and chemical agents*

For artificial transmission, experimental knowledge about the duration of the viability period of an infectious agent is of practical importance. At the time these investigations were undertaken the only available records on the resistance of *B. besnoiti* to the abovementioned agents were those of Cuillé, Chelle & Berlureau (1936) and Cuillé & Chelle (1937). For their tests they employed bovine blood collected during the febrile phase of the disease. The recipients were cattle obtained from a non-enzootic bovine besnoitiosis area in France. Fresh blood was administered within a few minutes after its withdrawal, while citrated blood was stored at room temperature for either five or 24 hours before injection. The inocula were administered intravenously. The experimental observations are listed in the subjoined Table 2.

In interpreting their results, Cuillé *et al.* (*loc. cit.*) assumed that a typical reaction in a recipient served as a criterion for viability of the infectious agent, and concluded that the survival period of *B. besnoiti* in citrated blood was five hours. A possible development of a mild or an inapparent infection in the test animals was not taken into account, and consequently neither immunity tests nor histological examination of skin sections were undertaken. It will be seen from Table 2 that in one of the two recipients that received one litre of citrated blood stored for 24 hours at room temperature, a " bout of fever " appeared 15 days after the injection, while the other

animal failed to show any clinical symptoms. A similar observation was made on the two recipients that received the suspension of emulsified cysts. One animal developed a " bout of fever " 15 days later, while the other showed no symptoms.

TABLE 2

Duration of viability period of B. besnoiti *as determined by Cuillé* et al. (1936, 1937

Recipient (ox)	Inoculum	Quantity in ml. administered i.v.	Maintained at	Period of Storage	Result
A	Fresh infective bovine blood	100·0	38° C	Few minutes	Reacted and recovered
B	Fresh infective bovine blood	150·0	38° C	Few minutes	Reacted and recovered
C	Infective bovine blood containing 5·0 gm. sodium citrate per 1,000 ml.	1,000	20° C	5 hours	Reacted and died
D	Infective bovine blood containing 5·0 gm. sodium citrate per 1,000 ml.	1,000	20° C	24 hours	A bout of fever 14 days later
E	Infective bovine blood containing 5·0 gm. sodium citrate per 1,000 ml.	1,000	20° C	24 hours	No reaction

The writer is of the opinion that Cuillé *et al.* (*loc. cit.*) were not justified in concluding that the infectious agent failed to remain alive for periods longer than five hours. This assertion is based on his own experience (Pols, 1954). In these tests two oxen each received one litre of blood collected from two donors which were showing fever. One of the recipients developed a thermal reaction six days later, and the other no clinical symptoms at all. Histological examination of skin section prepared three weeks later revealed typical *Besnoitia* cysts in both animals. Consideration of these results suggests that, in the tests conducted by Cuillé *et al.* (*loc. cit.*), the infectious agent survived for periods longer than five hours.

For the present studies on the duration of the viability period of *B. besnoiti in vitro*, rabbit blood showing a microscopic infection was employed. The different chemical agents, the various temperatures and the periods to which the infectious agent was exposed, and the rabbits used for determining the fate of the parasite, are listed in the appended Table 2 and in Table A of the Appendix. For these tests either 4·0 or 5·0 ml. of blood administered by the intraperitoneal route, and in one instance 10·0 ml. injected subcutaneously, were employed as inocula. Rabbits employed for the serial passage of the strain were used as donors, while the recipients acted as controls. The latter animals all reacted promptly to besnoitiosis after the intraperitoneal injection of fresh citrated blood. In some instances, where recipients failed to react to the treated blood, immunity tests were performed in order to exclude the possibility of an inapparent infection.

It will be noticed from the tests listed in Table 3 that exposure of infective blood to either potassium oxalate or heparin for a period of 30 minutes at room temperature had no influence on *B. besnoiti*. Edington's solution, commonly used as a preservative of viruses, had no immediate action but it killed the organism within 90 minutes after exposure. At room temperature the infectious agent in sodium citrate remained potent for periods of up to 48 hours but not after 96 hours. In citrated blood, stored for 24 hours at 37° C, the organism died. Blood held at +4° C for either 72 or 96 hours remained active. The end point of the viability period of *B. besnoiti* in citrated blood maintained at +4° C still needs to be determined. Further tests showed that the parasite did not survive when stored at −76° C for 12 days, and for 24 and 30 months, and at −18° C for 30 days.

The addition of penicillin and streptomycin to citrated blood did not improve the keeping qualities of the infectious agent. On the contrary, comparisons between the duration of the viability periods of the organism in citrated blood with that in citrated blood to which the two abovementioned antibiotics had been added, indicated that the latter products had a detrimental influence on vitality. When held at 20° C for 48 hours, and at +4° C for 96 hours the organism died, but when maintained at +4° C for 72 hours it remained alive. These results suggest that the detrimental influence of the antibiotics is retarded at a low temperature of +4° C, and hastened at a higher temperature of 20° C.

TABLE 3

Resistance of B. besnoiti *to physical and chemical agents*

No. of Rabbits	Stored Blood			No. that reacted	No. that died
	Time	Temperature °C	Anticoagulant		
8	0	39·0	Sodium citrate	8	3
2	1·5 hours	20·0	Sodium citrate	2	0
2	24 hours	20·0	Sodium citrate	2	0
1	24 hours	37·0	Sodium citrate	0	0
1	48 hours	20·0	Sodium citrate	1	0
1	72 hours	+ 4·0	Sodium citrate	1	1
1	96 hours	20·0	Sodium citrate	0	0
1	96 hours	+ 4·0	Sodium citrate	1	1
2	30 days	− 18·0	Sodium citrate	0	0
2	12 days	− 76·0	Sodium citrate	0	0
2	24 months	− 76·0	Sodium citrate	0	0
2	26 months	− 76·0	Sodium citrate	0	0
1	30 months	− 76·0	Sodium citrate	0	0
2	0	39·0	Sodium citrate together with *penicillin and *streptomycin	2	1
2	48 hours	20·0	Sodium citrate together with *penicillin and *streptomycin	0	0
1	72 hours	+ 4·0	Sodium citrate together with *penicillin and *streptomycin	1	1
1	96 hours	+ 4·0	Sodium citrate together with *penicillin and *streptomycin	0	0
1	0·5 hours	20·0	Potassium oxalate	1	1
1	0·5 hours	20·0	Heparin	1	1
2	0	39·0	*Edington's fluid	2	1
2	1·5 hours	20·0	*Edington's fluid	0	0

* See Table A of Appendix.

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Consideration of these results shows that the duration of the survival periods of *B. besnoiti*, as determined by the present tests, is very much longer than ascertained previously by Cuillé *et al.* (*loc. cit.*). It has also become apparent that rabbits are better suited for these tests than cattle, but that the determination of end points of the viability periods in different circumstances would entail the use of an extremely large number of animals over a long period. The limited time available did not permit conducting detailed systematic investigations, and hence studies were confined to establishing a practical and reliable method for the preservation of the infectious agent *in vitro*. The fact that *B. besnoiti* in citrated blood survives for four days at a temperature of +4° C, makes it possible to submit viable parasites from any part of the enzootic area in Transvaal to the central laboratory at Onderstepoort for biological studies.

(*g*) *Biological properties*

Besnoit & Robin (1912) established that *B. besnoiti* spores contain a toxin highly fatal to rabbits but to which guinea pigs and rats are refractory.

Whether or not immunologically different strains occur in nature still needs to be determined. Frenkel (1953 a), who tested sera from naturally infected cattle from South Africa, found that there is no measurable antibody content using *B. jellisoni* as antigen in the dye-test developed by Sabin & Feldman (1948).

(*h*) *Maintenance of the* B. besnoiti *strain*

The successful transmission of *B. besnoiti* to rabbits by means of blood derived from an affected ox during the febrile stage of the disease, offered a convenient method for maintaining the " Fuls " strain under laboratory conditions (Pols, 1954 a, b). During the course of the 125 serial passages it became apparent that several breeds of rabbits, and that both sexes, are equally susceptible. Cardiac blood from rabbits, when blood smears of donors showed a microscopic infection, was used as inoculum. On a few occasions blood from the marginal ear vein was employed.

During the first four generations the inoculum was administered by either the intravenous (one case, 3·0 ml.), intraperitoneal (three cases, 2·5, 3·0, 5·0 ml.) or subcutaneous route (three cases, 2·5, 10·0, 10·0 ml.). The ensuing reactions were characterised by a marked variation in the duration of the incubation periods. In the former rabbit it was six days, in the next three it varied from eight to 12 days, and in the latter three recipients it varied from 13 to 16 days.

From the fifth generation onwards the intraperitoneal route was employed as the standard method. The doses of blood used varied from 2·0 to 6·0 ml. Although no difference in the type of reactions followed this modification, a standard dose of 5·0 ml. of citrated blood was used from generation 25 onwards.

A total of 440 rabbits was employed for the 125 serial passages. Of these, 11 animals died from causes other than besnoitiosis, 352 developed typical reactions accompanied by microscopically demonstrable parasites, 68 only showing hyperthermia and/or subcutaneous swellings, and nine failed to reveal any symptoms. Neither subinoculations nor immunity tests were undertaken in the latter group of rabbits, and hence it is impossible to state whether or not an inapparent infection had occurred. Consideration of these observations makes it apparent that 20 per cent of recipients could not be relied upon as competent reservoirs for the maintenance of the " Fuls " strain, and that at least three rabbits are required for each serial passage.

(*i*) *Classification*

The names assigned to various species of Protozoa of the alimentary tract of the horse, domestic ruminants, armadillos, marsupials and certain snakes, included in either the genera *Eimeria* Schneider 1875; *Sarcocystis* Ray Lankester 1882; *Globidium* Flesch 1883; *Balbiania* Blanchard 1885; *Gastrocystis* Chatton 1910; *Ileocystis* Gilruth & Bull 1912; *Lymphocystis* Gilruth & Bull 1912; or *Haplogastrocystis* Chatton 1912, of those occurring in the cutis, subcutis, aponeuroses, sclera and mucous membrane of the upper respiratory tract of cattle and horses, included in either the genera *Sarcocystis*, *Globidium* or *Besnoitia* Henry 1913; and of the one found in the periosteum and tendons of the reindeer and the other encountered in the connective tissue of organs of the oppossum, included in the genus *Fibrocystis* Hadwen 1922, have long been the subject of discussion, much of which hopelessly intermixed zoological and nomenclatorial considerations.

Before discussing the classification of the parasites responsible for besnoitiosis, it will be necessary to give a brief historical review of the investigations on the abovementioned micro-organisms during the last 12 decades. In doing so it is hoped that the reader will gain a clearer concept of the events that led to the confusion in the nomenclature, and of the investigations that eventually permitted the correct identification of the *Besnoitia* spp. responsible for bovine, equine and murine besnoitiosis. Recent investigations by Frenkel (1953 a, 1955) and Pols (1954 a, b) have shown that the genus *Besnoitia* Henry 1913, is morphologically closely related to the genus *Toxoplasma* Nicolle & Manceaux 1909. In fact, the present investigations have shown that the microscopic identification of the two genera is based chiefly upon the recognition of the host cells. Members of the genus *Besnoitia* are harboured temporarily by monocytes and permanently by histiocytes, while the heteroxenous *Toxoplasma* sp. chiefly parasitizes cells of the reticulo-endothelial system, macrophages, monocytes and endothelial cells.

Miescher (1843) discovered the Sarcosporidia which Kuhn (1865) named *Synchitrium miescherianum* in the muscle fibres of a mouse. As this organism evidently did not belong to this genus, Ray Lankester (1882) included it in the genus *Sarcocystis*, while Blanchard (1885) named it *Sarcocystis muris*. Th. Smith (1893) found cysts, varying from 300 to 400 microns in diameter, which contained numerous merozoites in the mucous membrane of the small intestine of cattle in the United States of America. He concluded that the parasite was probably related to the Sarcosporidia. Marotel (1907), who found similar structures in the intestine of cattle in France, suggested that they represented the schizogonous phase of *Eimeria zürnii* (Rivolta, 1878). Bugge (1910) encountered pin-head sized cysts in the intestine of a calf in Germany, and suggested that they were immature stages of either coccidia or gregarines.

Besnoit & Robin (1912) discovered a parasite in the skin of cattle from the Pyrenees. It was named *Sarcocystis besnoiti* by Marotel (1912). The following year Henry (1913) created the genus *Besnoitia* and referred to the cutaneous parasite as *Besnoitia besnoiti* (Marotel 1912). Nöller (1920) revised the generic nomenclature of the genera *Globidium*, *Gastrocystis*, *Ileocystis*, *Lymphocystis*, *Haplogastrocystis* and *Besnoitia*. He retained the genus *Globidium*, and rejected the remaining generic names on the grounds that they did not possess any distinct, characteristic, morphological features. He concluded without justification that the cyst-like structure found in the intestine of cattle is identical with the cutaneous form, and included it under the name *Globidium besnoiti* (Marotel 1912).

Müller (1914) encountered cysts varying from 120 to 300 microns in size, harbouring numerous spores, in the intestine of cattle from Germany. He considered these structures to be schizonts of a coccidia. Railliet (1919), who revised the literature dealing with intestinal cysts, proposed that the parasite be named *Gastrocystis smithi* as there was no evidence that it was related to *E. zürnii*. Bruce (1921) demonstrated cysts in the small intestine of a calf in Canada. In one of the cysts he found merozoites, and concluded that these bodies were schizonts. In Holland Van Nederveen (1921) saw globular structures in the intestine of a calf, which he identified as *Gastrocystis smithi* Railliet 1919. In England Pillers (1928) encountered in the intestine of a bull cysts which he named *Globidium smithi*. Hassan (1935) described cysts of up to 1,000 microns in size containing numerous spores 13·0 microns in length and from 2·0 to 2·5 microns in width, in the mucous membrane of the pyloric end of the abomasum, and in that of the ileum of Indian cattle. As the parasitic cysts had not been described previously in the abomasum of cattle, he considered this parasite to be a new species, and proposed the name *Globidium fusiformis*. In the United States of America, Boughton (1942) observed that cysts in the mucosa of the small intestine of cattle occurred in association with coccidial infections, especially those of *Eimeria bovis* Züblin 1908. He also demonstrated the appearance of *E. bovis* oocysts five days after feeding material containing cyst-like structures to a week-old calf. From these observations he concluded that the cyst-like bodies were schizonts of one or more species of bovine coccidia, *E. bovis* being the most likely in the material observed by him. These investigations were followed by well planned biological and histological studies of Hammond, Bowman, Davis & Simms (1946), who confirmed the conclusions of Boughton (*loc. cit.*). They transmitted E. *bovis* to a series of calves and showed that the schizonts developed in the small intestine, while the sexual phase was usually found in the caecum and colon, except in severe infections. In these circumstances, sexual stages also appeared in the distal end of the small intestine.

The work conducted by Boughton (*loc. cit.*) and Hammond *et al.* (*loc. cit.*) was indeed an important landmark in the studies of bovine eimeriosis, globidiosis, and besnoitiosis. It brought forward convincing evidence that the intestinal parasite of Smith (1893), identified as *E. bovis*, and the cutaneous organism originally known as *Besnoitia besnoiti*, are not identical as suggested by Nöller (1920), who referred to them as *Globidium besnoiti*. The identity of the parasites described as *Gastrocystis smithi* Railliet 1919, *Globidium smithi* Pillers 1928, and *Globidium fusiformis* Hassan 1935, still needs to be determined. It is possible that the schizonts seen in the former two parasites are those of *E. bovis* (Hammond *et al.*, 1946).

Hammond *et al.* (*loc. cit.*), who considered the life-cycle of *E. bovis*, raised the question whether this parasite is a true *Eimeria* sp. They pointed out that the asexual stage of the life history of *E. bovis* is different from the classical description of eimerian coccidia in parasitizing cells of tissue other than epithelial, in its location in the centre of a villus, in the large number of merozoites produced by a single schizont, and in the occurrence of a single generation of schizonts. (It is not clear why the host cell specificity should be used as a criterion for the classification of coccidia. A characteristic feature of *Eimeria necatrix* Johnson 1930, is the development of a very large generation of schizonts in the sub-epithelium of the intestine of fowls). In an attempt to identify the generic status of *E. bovis* correctly, they compared the life-cycle of this parasite with that of other protozoa which also show large cysts in the course of their development. They stated that a " series of investigations by different workers had brought to light a pattern of development involving *Globidium* (the cystic stage of the parasite) and ending with *Eimeria*-type oocysts

in the camel (Henry & Masson, 1932; Enigk, 1934; Naville, 1936), armadillo (Reichenow & Carini, 1937) and horse (Reichenow, 1940, Hemmert & Halswick, 1943)." In these cases the *Globidium* stage is represented by the macrogametocyte and microgametocyte, and perhaps also by the schizont, although this stage was not clearly worked out. The oocysts are usually large (about 75 microns long), and have a thick outer membrane around the oocyst proper. Henry & Masson (1932) suggested that a certain group of species of *Globidium*, including *G. leuckarti* in the horse, *G. faurei* (= *G. gilruthi*) in the intestine of sheep, *G. cameli*, in the camel, and *G. besnoiti* in the small intestine and skin of cattle, be defined as coccidia whose endogenous reproductive stages reach a large size, and large oocysts with thick walls. The group was to be placed in coccidia, near or in the genus *Eimeria*. (It has already been pointed out above that there is not sufficient reason for grouping together the skin and intestinal forms of the so-called *Globidium* of cattle). Hammond *et al.* (*loc. cit.*) came to the conclusion that although it includes a *Globidium* stage, it does not fit into the pattern of the forms found in the camel, horse and armadillo. They arrived at the final conclusion " that the question of the systematic position of *E. bovis* can be better taken up when the life history of other species of coccidia in cattle, sheep and other hosts have been worked out in detail ".

Reichenow (1953) adopted a less critical attitude towards the classification of the genus *Eimeria* and closely allied parasites. He concluded that the available information on the life-cycle of several of the *Globidium* species was adequate to incorporate them as a subgenus of the *Eimeria*. The subgenus *Globidium* Flesch 1883, he defined so as to incorporate eimeria-like parasites situated in the sub-epithelial tissues of the intestine. Their developmental stages, especially the schizonts and microgametocytes, attain an exceptionally large size, thereby causing a marked hypertrophy of the host cell. The oocysts are extremely large, and are enveloped by two capsules of which the external one is thick, granular in structure and with a wrinkled surface. Reichenow (*loc. cit.*) was aware that this definition does not embrace the features of all the species that he intended to include in the subgenus *Globidium*, and, therefore, stressed that there is no sharp demarcation between the *Globidium* and *Eimeria s. str.* In the case of *E. bovis*, agamogony is typically that of a *Globidium*, while gamogony conforms with that of the *Eimeria s. str.* In his classification he referred to this parasite as *Eimeria* (*Globidium*) *bovis* (Züblin 1908) (Syn. *Eimeria smithi* Yakimoff & Galouzo 1927).

It has already been mentioned in this discussion that a great deal of confusion exists in the nomenclature of the parasite, causing cutaneous and subcutaneous lesions in cattle discovered by Besnoit & Robin (1912). Both investigators realized that it was a new parasite, and suggested that it might be a *Sarcocystis* sp. It was named *Sarcocystis besnoiti* by Marotel (1912). Since then, the generic and specific nomenclatures of *S. besnoiti* have undergone several changes. Jellison (1956), who gave a comprehensive account on the nomenclature, brought forward evidence that Henry (1913) used the generic name *Besnoitia*, used the combination *Besnoitia besnoiti*, and credited the authorship of this species to Marotel. Jellison (*loc. cit.*) pointed out that, although Henry (1913) believed that Brumpt (1913) had established the genus *Besnoitia* in the second edition of his Précis de Parasitologie, in preparation, scrutiny of this publication subsequently revealed that Brumpt had not proposed the generic name. On the contrary, Brumpt referred to the bovine cutaneous parasite as *Gastrocystis Robini* de la vache (Besnoit & Robin) on page 105, and as *Gastrocystis besnoiti* (Marotel) on page 113.

Franco & Borges (1916) formally proposed the generic name *Besnoitia*, as they believed that the method of presentation by Henry (1913) did not comply with the nomenclatorial rules. In the Zoological Record of 1916 and in the Nomenclator Zoologicus by Neave (1939), Franco & Borges (1916) were credited with the authorship of the generic name *Besnoitia*.

It has been mentioned before that Nöller (1920) revised the generic nomenclature of the genera *Globidium*, *Ileocystis*, *Lymphocystis*, *Haplogastrocystis* and *Besnoitia*, that he retained the genus *Globidium*, that he rejected the remaining generic names, that he concluded, without justification, that the cyst-like structures found in the intestine of cattle are identical with the cutaneous forms, and that he included them under the name *Globidium besnoiti* Marotel 1912. In subsequent publications it will be seen that the name *Globidium besnoiti* was used by Wenyon (1926), Doflein & Reichenow (1929), Henry & Masson (1932) and Neveu-Lemaire (1943). Brumpt (1927, 1949) also followed Nöller (1920) in the change of the generic assignment, but erred again in crediting the authorship to Besnoit and Robin instead of to Marotel, for he listed *Globidium besnoiti* de la vache (Besnoit & Robin).

Babudieri (1932), who revised the classification of the Sarcosporidia, came to the conclusion that the cutaneous parasite of cattle is a distinct species, and is not related to the intestinal parasite. He transferred the cutaneous parasite to the genus *Besnoitia*, and listed it as *B. besnoiti* (Marotel 1912). In the revision of the classification of the *Globidium* spp., Reichenow (1953) came to the same conclusion. He referred to the cutaneous parasite as *B. besnoiti* (Marotel, 1912), and to the intestinal parasite as *Eimeria* (*Globidium*) *bovis* (Züblin 1908).

Up to this stage no studies had been conducted on the life-cycle of the bovine and equine *Besnoitia* spp. The mode of reproduction became evident when Frenkel (1953 a) described *Besnoitia jellisoni* in a white-footed mouse (*Peromyscus maniculatus*) (Wagner) Glooger. He established and maintained this parasite in laboratory animals. Microscopic observations revealed that this protozoon multiplies by binary fission, and that it bears a morphological resemblance to *Toxoplasma gondii* which also reproduces in the same fashion. Pols (1954 a, b) transmitted *B. besnoiti* to rabbits, and maintained it successfully by serial passage in this animal. Studies showed that the bovine parasite also reproduces by simple binary fission. These observations disproved the assumption of Nöller (1920) that the *Besnoitia* sp. multiplies by schizogony as had been determined for the intestinal parasite.

From this historical review it becomes apparent that no less than three generic names, namely *Gastrocystis*, *Globidium* and *Eimeria*, had been assigned to the intestinal protozoon *Eimeria* (*Globidium*) *bovis*, and that four generic names, namely *Sarcocystis*, *Besnoitia*, *Gastrocystis* and *Globidium* had been allotted to the cutaneous protozoon *B. besnoiti*. Jellison (1956), who scrutinized the nomenclature of the genus *Besnoitia* and of the species *B. besnoiti* and *B. jellisoni*, stated that " he offers no opinion on whether or not these two species deserve the distinction of a genus separate from *Gastrocystis*, *Globidium* or *Sarcocystis* ". Comparison between the habitat, morphology, life-cycle and host-relationship of the *Gastrocystis* spp., *Globidium* spp., *Sarcocystis* spp. and *Besnoitia* spp. leaves no doubt that *Besnoitia* is a distinct genus, and that the two species *B. besnoitia* and *B. jellisoni* are, according to available evidence, stenoxenous parasites, a feature not possessed by members of the remaining three genera.

In order to obtain clarity about the identity of the two parasites of the horse, included in the genus *Globidium*, it will be necessary to review the relevant literature. Flesch (1883) found a parasite in cysts, 300 by 170 microns in size, in the villi of the small intestine of a horse in Germany. He named it *Globidium leuckarti*. This organism was again encountered by Hobmaier (1922) and Kupke (1923) in Europe. The latter investigator found, in addition to the cysts harbouring merozoites, large unripe oocysts enveloped in a thick capsule in the villi of the small intestine. According to Neveu-Lemaire (1943), Henry & Masson (1922) found cysts, varying from 230 to 400 microns in diameter, which harboured numerous organisms, in the cutis and subcutis of a horse in the vicinity of Rheims in France. Since the spores within the cutaneous cysts showed some resemblance to the intestinal parasite described by Flesch (1883), he identified the cutaneous protozoon as *Gl. leuckarti*. [It is obvious that Henry & Masson (*loc. cit.*) adopted the same scheme as Nöller (1920).] Bennett (1927) described several cases of cutaneous sarcosporidiosis in horses from the Sudan. In a subsequent publication (Bennett, 1933) he stated that Wenyon had identified the causal agent as a *Globidium* sp.

Consideration of this account makes it apparent that two distinct types of globidiosis, namely the intestinal and the cutaneous forms, occurred in horses, and that according to Henry & Masson (1922) *Gl. leuckarti* was responsible for both diseases. Babudieri (1932), who critically reviewed the classification of the class Sarcosporidia Bütschli 1882, concludes that *Gl. leuckarti*, originally described as the causal agent of intestinal globidiosis, is morphologically distinct from the parasite responsible for equine sclerodermatitis. Histological examination revealed that the equine cutaneous parasite, with its cystic membrane, is morphologically indistinguishable from *B. besnoiti*. He considered that the cutaneous parasite described by Bennett (1927) is specific for the horse, proposed that it be included in the genus *Besnoitia*, and listed it as *Besnoitia bennetti* Babudieri 1931. Bennett (1933), who described the presence of free spores in the areolar tissue of a horse and the development of cysts harbouring the causal agent, comes to the conclusion that " in spite of the increase in the number of parasites, there is no suggestive arrangement of spores, or any other appearance to indicate that they have undergone but the simplest form of division ". From these observations he suggests " that the organism now known as *Globidium besnoiti*, including in this species the parasite in the Sudan, is probably not a globidium or alternatively that the definition of the genus *Globidium* needs redrafting. The decision must be left to those better qualified to make it, but it seems that the species *Sarcocystis besnoiti* might still be considered good ". Enigk (1934), who carefully studied the life-cycle of *G. cameli* and that of *Gl. leuckarti*, came to the conclusion that the equine cutaneous parasite described by Bennett (1933) is not a *Globidium* sp.

The proposal of Babudieri (*loc. cit.*) was not generally accepted. Bennett (1937) still referred to the cutaneous parasite as a *Globidium* sp. Neveu-Lemaire (1943) continued to consider the intestinal and cutaneous parasites as identical, listed it as *Gl. leuckarti*, and retained it in the sub-family Eimerinae Henry & Masson 1932. Although Reichenow (1953) did not accept Babudieri's classification of the class Sarcosporidia, he agreed that the correct name for the cutaneous parasite is *B. bennetti*.

Investigations conducted on the life-cycle of the intestinal parasite (*Gl. leuckarti*) by Reichenow (1940) revealed the presence of immature oocysts in the faeces of a horse and a donkey. After 14 days the oocysts showed four sporoblasts, and seven days later the ensuing sporocysts each harboured two sporozoites. These obser-

vations caused this investigator to include the parasite in the genus *Eimeria*. Studies by Hemmert-Halswick (1943) in Germany, and Hiregaudar (1956) in Pakistan confirmed the previously observed eimerian features of the intestinal parasite *Gl. leuckarti*. Reichenow (1953) listed this organism as *Eimeria* (*Globidium*) *leuckarti* (Flesch 1883).

From this historical review it becomes apparent that the two generic names *Globidium* and *Eimeria* had been assigned to the equine intestinal parasite, that the former genus received a sub-generic status, and that it was thereupon included in the genus *Eimeria*. Three generic names, *Globidium*, *Sarcocystis* and *Besnoitia*, had been allotted to the cutaneous parasite. The former two names were rejected while *Besnoitia* was accepted as the correct generic name. Detailed studies on the life-cycle of the cutaneous protozoon have not yet been carried out but there is every reason to believe that the conclusion of Bennett (1933) that the parasite divides by binary fission, will be confirmed.

Scrutiny of the literature on the abomasal and intestinal parasites of sheep and goats has shown that confusion still exists about the identity of *Gl. gilruthi* Chatton 1910. Maske (1893) found cysts up to 1,000 microns in diameter, harbouring numerous spores, in the abomasal mucous membrane of sheep in Germany. He considered this parasite to be related to the gregarines. Moussu & Marotel (1902), who encountered this abomasal parasite in sheep from France, believed that it represented a developmental phase of *Eimeria faurei* Moussu & Marotel 1901. Gilruth (1910) recorded the occurrence of abomasal cysts, harbouring blastophores and spores, in Australian sheep. Chatton (1910) encountered the same parasite in the abomasum and small intestine of sheep and goats from Australia. He first named the parasite *Gastrocystis gilruthi* in 1910, and during the same year included it in the genus *Globidium*. Neveu-Lemaire (1943) listed the protozoon as *Globidium faurei* (Moussu & Morotel 1902), and incorporated it in the sub-family Eimerinae. Reichenow (1953), on the other hand, provisionally included this organism in the genus *Eimeria* on the assumption that the abomasal and intestinal cysts represent the schizogonous phase, and that the gamogonic phase, which has not yet been determined experimentally, probably develops in the distal portion of the small intestine. He suggested that *Eimeria intricata* Spiegl 1925, which possesses a relatively large thick-walled oocyst, probably represents the sporogonic phase in the life-cycle of *Gl. gilruthi*. He tentatively transferred this protozoon to the genus *Eimeria*, included it in the subgenus *Globidium*, and listed it as *Eimeria* (*Globidium*) *gilruthi* (Chatton 1910) (syn.: *Gastrocystis gilruthi* Chatton 1910).

Investigations on this group of parasites continued. In a dromedary (*Camelus dromedarius* Lin.) submitted at the Alfort Veterinary College in France, Henry & Masson (1932) discovered, at autopsy, mature macrogametocytes and a very large, thick-walled, immature oocyst in the mucosa of the small intestine. They named the parasite *Gl. cameli*. Enigk (1934), who worked in Germany, found the same parasite not only in the jejunun and ileum, but also in the caecum of a camel (*Camelus bactrianus* Lin.) which originally came from Uralsk. He described immature and mature schizonts, microgametocytes and large unripe oocysts. The mature forms of the former two stages measured 200 by 150 microns. Reichenow (1953) cast doubt on Enigk's interpretation of the agamonic phase in which minute merozoites 2·0 microns in length appeared. He suggested that the alleged schizonts actually represented developing microgametocytes. Consideration of the nature of the gamogonic and sporogonic stages caused Reichenow (*loc. cit.*) to transfer *Gl. cameli* to the genus *Eimeria*, to include it in the subgenus *Globidium*, and to list it as *Eimeria* (*Globidium*) *cameli* Henry & Masson 1932.

In Brazil, *Globidium*-like parasites were found in two armadillo species. *Gl. travassosi* Da Cunha & Muniz 1928, was found in the subepithelial tissues of the small intestine of the six-banded armadillo [*Dasypus sexcinctus* Lin. = *Euphractus sexcinctus* (Lin).] The developmental stages appeared in markedly hypertrophied host cells. A detailed description of the gamogonic stages and of the immature and mature oocysts was given by Reichenow & Carini (1937). They stated that the agamogonic phase was represented by a single schizont, 100 by 84 microns in size, containing numerous merozoites. They expressed the opinion that the figures quoted did not represent the actual dimensions of the schizont, as the single section available had been cut near the margin, and that the maximum size was in all probability much greater. Reichenow & Carini (*loc. cit.*) suggested that this parasite may actually be identical with *Gl. tatusi* Da Cunha & Torres 1926, described from the nine-banded armadillo [*Dasypus novemcinctus* (Lin.) = *Tatus novemcinctus* Lin.)] The mature schizonts of this parasite, the only stage seen, can attain the tremendous size of 2,100 by 500 microns. Should this prove to be correct, then *Gl. travassosi* becomes synonymous for *Gl. tatusi.* Reichenow & Carini (*loc. cit.*) transferred *Gl. travassosi* tentatively to the genus *Eimeria*, included it in the subgenus *Globidium*, and listed it as *Eimeria* (*Globidium*) *travassosi* Da Cunha & Muniz 1928.

For the sake of completeness the remaining protozoa included in the genus *Globidium* will be considered in this review. They comprise parasites of Australian marsupials and European snakes. It will be noticed that only cysts containing either blastophores and/or spores which may represent schizonts of an *Eimeria*, were described.

Before discussing the intestinal parasites of the marsupials, it must be mentioned that Reichenow (1953) stated that the knowledge about their developmental cycles is incomplete, and that their systematic status still needs to be determined.

Blanchard (1885) found sarcosporidia-like cysts, varying from 710 to 1,230 microns in length and 510 to 950 microns in width, and harbouring kidney-shaped spores 9·8 to 12·0 microns in length, in the connective tissue of the small intestine of a kangaroo [*Macropus penicillata* (Gray) = *Petrogale penicillata* (Gray)]. According to Wenyon (1926), he named the parasite *Sarcocystis mucosae*, while Reichenow (1953) stated that he named it *Balbiania mucosa.* Coutelen (1933), who re-examined the intestinal globules, found that the cysts were chambered, and therefore concluded that the parasite was a *Sarcocystis* sp.

Gilruth & Bull (1912) recognised two types of cysts in a kangaroo which had died of haemorrhagic enteritis. The cysts were situated in the subepithelial tissues of the markedly inflamed small intestine. The large cysts, with a diameter varying from 70 to 334 microns occurred in groups situated in the deep layers of the mucous membrane. Single cysts, with a distinct membrane, appeared within hypertrophied cells. Free spores were not seen. They named the parasite *Ileocystis macropodis.* Gilruth & Bull (1912) created a new genus *Haplogastrocystis*, and changed the name *I. macropodis* to *Haplogastrocystis macropodis.* Nöller (1920), who revised the generic nomenclature, regarded, without justification, *Balbiania mucosa* as identical with *H. macropodis*, included them in the genus Globidium, and used the combination *Gl. mucosa* Blanchard 1885? as a possible correct name.

The smaller cysts were demonstrated in great numbers in the connective tissue of the mucosa. When mature, they had a diameter of 8·4 microns and were filled with spores 5·5 microns long and from 2·0 to 2·5 microns broad. The cystic wall was extremely thin, and contained a compressed nucleus. It appeared that the host

cell was a mononuclear leucocyte. This parasite was named *Lymphocystis macropodis* Gilruth & Bull 1912. This protozoon was not included in the genus *Globidium* by Nöller (1920).

In two wallabies (*Petrogale* sp.) which had died of enteritis, and in an apparently healthy one, Gilruth & Bull (1912) found cysts, arranged in groups, in the submucosa of the small intestine. The cysts had a multilocular structure, the wall of which consisted of two membranes. They contained numerous blastophores which gave rise to spores. Chatton (1912) suggested that this parasite be included in the genus *Haplogastrocystis*. Nöller (1920) listed it as a *Globidium* spec. Gilruth & Bull 1912.

Wenyon & Scott (1925) and Triffit (1926) described parasites in the black wallaby [*Macropus ualabatus* (Lesson & Gurnior) = *Macropus bennetti* Lesson & Gurnior] which resembled *I. macropodis* and *L. macropodis*. They were, however, unable to determine whether these parasites were related. In addition they found that the muscle fibres of the intestine contained elongate vacuolic spaces filled with spores, which appeared very similar to those of the *Lymphocystis* type. Whether this, again, was a species of *Sarcocystis* or another stage of *Lymphocystis* remained obscure. Wenyon & Scott (*loc. cit.*) concluded that if three parasites were represented, then, in the portion of the intestine which they examined, four distinct species occurred, as an *Eimeria* sp. was also present in the epithelium. In conclusion they suggested that the parasite *S. mucosae* Blanchard 1885, might be identical with *I. macropodis*.

In a hairy nosed wombat [*Lasiorhinus latrifrons* (Owen) = *Phascolomys latrifrons* Owen] that died at Melbourne and which showed a chronic catarrhal papillomatose enteritis of the small intestine, Gilruth & Bull (1912) found a parasite which they named *Ileocystis wombati*. This protozoon occurred within round or oval thick-walled cysts, varying from 93 to 114 microns in size. The cysts were situated within the proliferating connective tissue of the hypertrophied villi and also within the glands of Lieberkühn. In structure it resembled *Gl. gilruthi*. Chatton (1912) suggested that this parasite should be included in the genus *Haplogastrocystis*, while Nöller (1920) transferred it to the genus *Globidium* and listed it as *Gl. wombati* Gilruth & Bull 1912.

The last *Globidium* species to be considered is *Gl. navillei* Harant & Gazal 1934, which has been recorded from two snakes, *Tropidonotus natrix* Lin. and *T. viprinus* Lin. Schizonts up to 500 microns in size appeared in the markedly hypertrophied host cells in the subepithelial tissue of the intestinal mucosa. Gamogonic stages and oocysts were not observed. The suggestion of Naville (1931) that these large schizonts represented the agamogonic phase of *Eimeria tropidonoti* Guyenot, Naville & Ponse 1922, was not accepted by Harant & Gazal (*loc. cit.*). In order to avoid an unnecessary nomenclatorial confusion, Reichenow listed the protozoon as *Gl. navillei* Harant & Gazal 1934.

The last genus that needs attention is *Fibrocystis* Hadwen 1922. The first species, *F. tarandi* Hadwen 1922, to be described was found in fibrous tissue, especially that covering the tendons and the periosteum of the reindeer (*Rangifer tarandi* Lin.). The cysts have a diameter of 100 to 450 microns, while the cystic wall consists of three layers enclosing numerous spores. This parasite causes " corn meal disease ", and when in the periosteum the cysts cause atrophy of the bones, which appear pitted. Wenyon (1926) suggested that it may be related to the *Sarcosporidia*, while

Babudieri (1932) was of opinion that it may be allied to the genus *Besnoitia*. The second species, *F. darlingi* (Brumpt 1915), of the opposum (*Didelphis* sp.) was originally included in the genus *Sarcocystis* by Brumpt (1915). It occurs in non-chambered oval cysts, varying from 1,500 to 2,000 microns in size, which are situated in the connective tissue of parenchymatous organs. The structure of the cyst and the habitat of the parasite, which differs from those of a typical *Sarcocystis* sp., caused Babudieri (1932) to transfer this parasite to the genus *Fibrocystis*.

Having surveyed in detail the complicated history of the nomenclature of certain Protozoa included in the general *Sarcocystis*, *Eimeria*, *Globidium*, *Balbiania*, *Gastrocystis*, *Ileocystis*, *Lymphocystis*, *Haplogastrocystis*, *Besnoitia* and *Fibrocystis*, and having indicated the criteria employed in their classification, the views expressed by several protozoologists about their status in the higher taxonomic categories will be submitted in chronological order.

Nöller (1920) stated that before the complete developmental cycle of a representative of a genus is not known in detail, it is impossible to arrange it in the classificatory system of the Protozoa. He reviewed the relevant literature up to the year 1918, and stressed that the taxonomic studies by several investigators had not brought forward a clearer concept about their identity. He regarded the assignment of various generic names to certain intestinal and cutaneous parasites as futile. He recommended that, for the sake of simplicity, the parasites be included in the genus *Globidium* Flesch 1883 and that the other generic names be regarded as synonyms. In his revised generic nomenclature he listed the following species, together with their synonyms, as:—

Globidium leuckarti Flesch 1883. (*Equus caballus* Lin.)

Globidium gilruthi Chatton 1910.
(*Ovis aries* Lin. and *Capra hircus* Lin.)
Synonym: *Gastrocystis gilruthi* Chatton 1910.

Globidium besnoiti Marotel 1912. (*Bos taurus* Lin.)
Synonyms: *Gastrocystis besnoiti* Marotel 1912,
Besnoitia nov. gen. Franco & Borges 1916.

Globidium mucosa Blanchard 1885? [*Macropus penicillata* (Gray)].
Synonyms: *Ileocystis macropodis* Gilruth & Bull 1912;
Haplogastrocystis nov. gen. Chatton 1912.

Globidium sp. Gilruth & Bull 1912. (*Petrogale* sp.)
Synonym:? *Sarcocystis macropodis* Gilruth & Bull 1912.

Globidium wombati Gilruth & Bull 1912.
[*Lasiorhinus latrifrons* (Owen)].
Synonyms: *Ileocystis wombati* Gilruth & Bull 1912.
Haplogastrocystis nov. gen. Chatton 1912.

Although the species *Lymphocystis macropodis* Gilruth & Bull 1912, was discussed by Nöller (1920) under *Gl. mucosa*, it was not included in the genus *Globidium*. It will also be noticed that the synonyms *Sarcocystis besnoiti* Marotel 1912, and *Gastrocystis robini* Brumpt 1913, are not listed as synonyms of *Gl. besnoiti*.

Wenyon (1926) referred to the abovementioned organisms as parasites of undetermined position. He stated that " there are a number of organisms which are usually grouped with the Cnidosporidia, though they do not show any of their characters. It is doubtful if some are Protozoa at all. The chief of these are: the Sarcosporidia, which are parasitic in the muscle fibres of vertebrates, and have the form of elongate chambered bodies filled with sickle-shaped spores; the closely related *Globidium* (cysts of Gilruth), which give rise to nodules in the mucosa of the stomach and intestine of ruminants and others; the Haplosporidia, which occur chiefly in invertebrates in the form of uninucleate or multinucleate cytoplasmic bodies are resistant spores; and the Serumsporidia, which are found in the body-cavity fluids of aquatic crustacea and larvae as small round cells which multiply by binary fission or schizogony. The Rhinosporidia, which produce nasal polypi, have been usually classed with the Haplosporidia, but Ashworth (1923) has shown conclusively that they are fungi, and not Protozoa, and this appears to be true of *Globidium* and possibly the Sarcosporidia ".

Wenyon (1926) considered that parasites included in the genus *Globidium* Flesch 1884 (date should be 1883), " are probably related to the Sarcosporidia, have the form of spherical cysts up to 5 millimetres in diameter embedded in the mucosa of the alimentary canal or skin of mammals. Each is enclosed by a membranous capsule, and when fully grown consists of spores which resemble those of the Sarcosporidia ".

Under the Sarcosporidia Bütschli 1882, Wenyon (1926) listed the following parasites:—

Globidium leuckarti Flesch 1884. (*Equus caballus* Lin.)

Globidium gilruthi Chatton 1910.
(*Ovis aries* Lin., and *Capra hircus* Lin.)
Synonyms: *Gastrocystis gilruthi* Chatton 1910;
Eimeria faurei Moussu & Marotel 1902.

Globidium besnoiti Marotel 1912. (*Bos taurus* Lin.).
Synonyms: *Sarcocystis besnoiti* Marotel 1912.
Besnoitia nov. gen. Franco & Borges 1916.

Globidium tatusi Da Cunha & Torres 1924.
[*Dasypus novemcinctus* (Lin.)]

Sarcocystis mucosae Blanchard 1885. (*Macropus* sp.).
Synonym:? *Ileocystis macropodis* Gilruth & Bull 1912.

Sarcocystis macropodis Gilruth & Bull 1912.
(*Petrogale* sp.).

Ileocystis macropodis Gilruth & Bull 1912.
[*Macropus penicillata* (Gray) and *M. ualabatus* Lesson & Gurnior.]
Synonym:? *Lymphocystis macropodis* Gilruth & Bull 1912.

Ileocystis wombati Gilruth & Bull 1912.
[*Lasiorhinus latifrons* (Owen)].

Lymphocystis macropodis Gilruth & Bull 1912.
[*Macropus penicillata* (Gray), and *M. ualabatus* (Lesson & Gurnior)].

Fibrocystis tarandi Hadwen 1922 (*Rangifer tarandus* Lin.).

Reichenow (1929) came to the conclusion that there is no convincing evidence that the parasites under discussion are Protozoa, and that only careful studies on their life-cycles would reveal their true identity. He stated that the views expressed by Chatton (1910) and Nöller (1920) on the gregarine-like development appeared to be more acceptable than those of other investigators who had assumed that these parasites belong to the Sarcosporidia. In this review (Reichenow *loc. cit.*) used the name *Gl. besnoiti* for both the intestinal and cutaneous parasites of cattle, and *Gl. leuckarti* for both the intestinal and cutaneous parasites of the horse. He gave a brief account of the intestinal parasites of the Australian marsupials, but refrained from mentioning their names. He listed the following species:—

Globidium leuckarti Flesch 1883. (*Equus caballus* Lin.).
Globidium besnoiti Marotel 1912, (*Bos taurus* Lin.).
Globidium gilruthi Chatton 1910.
(*Ovis aries* Lin. and *Capra hircus* Lin.).
Globidium tatusi Da Cunha & Torres 1926.
[*Dasypus novemcinctus* (Lin.)].

Babudieri (1932) carefully reviewed the literature of the Sarcosporidia and of the parasites under discussion. He came to the conclusion that the latter possessep characteristics which permitted their inclusion in the sub-class Sarcosporidia. In doing so he created two new orders and three new families. The revised classification is as follows:—

CLASS: Sporozoa Leuckart 1879.

SUB-CLASS: Sarcosporidia Balbiani 1885.

This sub-class comprises Sporozoa situated in striated and non-striated muscles and connective tissue of mammals, birds and reptiles. The parasites appear as banana-shaped or fusiform structures within cysts formed by either the host, the parasite or by the combined action of the host and parasite. The cystic cavity may or may not be chambered. The spores harbour a nucleus, usually granular and with a distinct nucleolus. In the middle of the spore metachromatic and siderophilic granules are visible.

ORDER: Sarcosporidia Bütschli 1882.

The order includes banana-shaped spores situated within cysts, which are either totally or partially of parasitic origin. Spores are provided with metachromatic granules and secrete a toxin known as sarcocystin. They are derived from proliferating pansporoblasts and sporoblasts situated along the margin of the cyst.

FAMILY: Sarcocystidae Babudieri 1932.

The family is represented by a single genus, *Sarcocystis*, which comprises Sarcosporidia situated exclusively in striated muscles. Cysts are elongated and sub-divided by septa, and seldom cause an inflammatory reaction.

GENUS: *Sarcocystis* Ray Lankester 1882.

S. miescheriana Kuhn 1865. (*Sus scrofa* Lin.).

In addition 21 *Sarcocystis* spp. occurring in either mammals, birds or reptiles are listed.

FAMILY: Fibrocystidae Babudieri 1932.

The family comprises Sarcosporidia of the connective tissue and non-striated muscle. Cysts are large and round, not subdivided by septa, and are surrounded by a thick fibrous capsule. The genus *Fibrocystis* is situated in the deep connective tissue, periosteum and connective tissue of parenchymatous organs, and rarely affects the host. The genus *Besnoitia* is situated mainly subcutaneously and is highly pathogenic.

GENUS: *Fibrocystis* Hadwen 1922.

F. tarandi Hadwen 1922. (*Rangifer tarandus* Lin.).

F. darlingi Brumpt 1915. (*Didelphis* sp.).

Synonym: *Sarcocystis darlingi* Brumpt 1915.

Besnoitia Franco & Borges 1916.

B. besnoiti Marotel 1912. (*Bos taurus* Lin.).

B. bennetti Babudieri 1931. (*Equus caballus* Lin.).

ORDER: Globidia Babudieri 1932.

The order comprises parasites with a few siderophilic granules in the spores, which do not secrete a toxin. Cysts are situated exclusively in the sub-mucosa of the alimentary tract. The host cell is a hypertrophied somatic cell, the nucleus of which has a marginal situation. The spores are derived from mulberry-like masses, the so-called blastophores.

FAMILY: Globididae Babudieri 1932.

The family includes two genera. The genus *Globidium* is characterised by multiple blastophores, and the genus *Ileocystis* by a single blastophore within the cyst.

GENUS: *Globidium* Flesch 1883.

G. leuckarti Flesch 1883. (*Equus caballus* Lin.).

G. gilruthi Chatton 1910. (*Ovis aries* Lin.).

G. besnoiti Marotel 1912. (*Bos taurus* Lin.).

G. tatusi Da Cunha & Torres 1926. [*Dasypus novemcinctus* (Lin.)]

G. macropodis Gilruth & Bull 1912. [*Macropus penicillata* (Gray), *M. bennetti* Owen, *Petrogale* sp.].

GENUS: *Ileocystis* Gilruth & Bull 1912.

I. macropodis Gilruth & Bull 1912. [*Macropus penicillata* (Gray)].

Reichenow (1953) briefly reviewed the classification of Babudieri (1932), and stressed that the taxonomy of this group of organisms is unsatisfactory as in many cases the available knowledge about their life-cycles is incomplete. He pointed out that members of the order Sarcosporidia are usually included in the single genus *Sarcocystis*, but that the incorporation of the genera *Fibrocystis* and *Besnoitia*

occurring in unilocular cysts, into this order is very problematic. However, he agrees that members of these genera are distinct from the *Globidium* spp. of the alimentary tract, as studies on the life-cycle of the latter, in most cases, had shown that they belong to the coccidia. It will be seen from Reichenow's revised classification that he retained the intestinal parasites of the marsupials and snakes in the subgenus *Globidium*, but that he refrained from listing their names in the genus *Eimeria* as there is no evidence that gamogony and sporogony occur in their developmental cycle.

Reichenow's revised classification of the genus *Globidium* is as follows:—

CLASS..... Sporozoa Leuckart.
SUB-CLASS... Telesporidia Schaudinn.
ORDER..... Coccidia Leuckart.
SUB-ORDER.. Eimeridea Léger.
FAMILY..... Eimeridae Léger emend.
GENUS..... *Eimeria* Schneider 1875.
SUB-GENUS... *Globidium* Flesch 1883.

Synonym: *Gastrocystis* Chatton 1910.

Eimeria (*Globidium*) *leuckarti* Flesch 1883. (*Equus caballus* Lin.).

Eimeria (*Globidium*) *cameli* Henry & Masson 1932. (*Camelus bactrianus* Lin. and *C. dromedarius* Lin.).

Eimeria (*Globidium*) *gilruthi* (Chatton 1910). (*Ovis aries* Lin. and *Capra hircus* Lin.).

Synonym: *Gastrocystis gilruthi* Chatton 1910.

Eimeria (*Globidium*) *travassosi* (Da Cunha & Muniz 1928). [*Dasypus sexcinctus* (Lin.)].

?*Eimeria* (*Globidium*) *tatusi* Da Cunha & Torres 1926. [*Dasypus novemcinctus* (Lin.)].

Synonym: ?*Eimeria* (*Globidium*) *travassosi* da Cunha & Muniz 1928.

Eimeria (*Globidium*) *bovis* (Züblin 1908) (*Bos taurus* Lin.)

Synonym: *Eimeria smithi* Yakimoff & Galouzo 1928.

Consideration of the investigations conducted during the last five decades makes it apparent that sufficient evidence has been brought forward to prove that *B. besnoiti* is neither a coccidium nor a sarcosporidium, and that the determination of its identity was largely dependent upon studies of the life-cycle. Bennett (1933) concluded from his histological studies that *B. besnoiti* of the horse (now known as *B. bennetti*) in all probability multiplied by the simplest form of division, namely binary fission. A profitable method of approach for these studies was offered by Cuillé, Chelle & Berlureau (1936) and Barrairon (1938), who showed that bovine besnoitiosis can be reproduced artificially in cattle by administering infective blood by either the subcutaneous or intravenous route. This was indeed another important landmark in the investigations on besnoitiosis, but it was left to Frenkel (1953 a, 1956) to finally disclose the vertebrate life-cycle and immunogenic properties of the genus *Besnoitia*. His studies on *B. jellisoni* not only revealed that this protozoon

reproduced by repeated binary fission, but that it is morphologically related to, though immunologically, distinct from, the heteroxenous parasite, *T. gondii*. Frenkel's observations were confirmed by Pols (1954 a, b), who showed that *B. besnoiti* is endowed with similar properties as *B. jellisoni*, and that the derivation of the cystic wall, which had given rise to a great deal of speculation, is of somatic origin.

Having surveyed in detail the complicated history of the nomenclature of the *Besnoitia* spp. and *Fibrocystis* sp., and having brought forward evidence that members of both genera are related to *T. gondii*, it will be necessary to give a brief review of the nomenclatorial history of the toxoplasms, as in recent years the classification has been modified.

The genus *Toxoplasma* is characterised by oval, crescentic or reniform parasites, 2·5 to 6·5 microns in length and 1·5 to 3·5 microns in breadth, possessing a single nucleus which is mostly central but sometimes nearer one end than the other. The ends of the body are usually pointed, one end usually being blunter and broader than the other. The parasites multiply by longitudinal binary fission, and gradually fill and distend the host cells, the reticulo-endothelial cells, mononuclear leucocytes, and more rarely polymorphonuclears. These aggregations are referred to as pseudocysts, are usually rounded, vary from 5 to 50 microns in diameter, and harbour up to one hundred or more parasites. The natural mode of transmission is obscure. It has been suggested that the infection is acquired by ingestion of food contaminated with infected faeces or some arthropod vector. Toxoplasms may be pathogenic to both naturally and experimentally infected animals, and the ensuing disease may have a fatal termination.

Species names have been assigned to a large number of toxoplasms recorded from mammals and birds. However, investigations by Carini (1909) and Manwell & Drobeck (1951) have shown that the parasites described from different mammalian hosts are not only morphologically but also immunologically identical. The fact that *T. gondii* will infect rabbits, guinea-pigs, dogs, mice, moles, pigeons and Java sparrows, in all of which naturally occurring *Toxoplasma* infections have been found, raises the question of the identity of various species (Wenyon, 1926). It thus appeared that convincing evidence had been brought forward that the genus *Toxoplasma* is represented by a single species, *T. gondii*.

Garnham (1950) modified this conception after comparing the morphology of *T. gondii* with that of the toxoplasma-like organisms encountered in naturally infected birds. He brought forward evidence that the mammalian and avian parasites are distinct, and created the genus *Atoxoplasma*, which comprises avian parasites from many parts of the world which are strictly host specific, non-pathogenic, possess a delicately granular cytoplasm not enclosed in a periplast, and a large diffuse nucleus with a tiny karyosome. *Atoxoplasma avium* (Marullaz, 1913) was selected as the type species. Laird (1959), who established *Atoxoplasma* parasites in several species of birds belonging to the family Zosteropidae in the South Pacific region, came to the conclusion that they are not host specific and stated that Lainson (1958) had demonstrated that the *Atoxoplasma* may be pathogenic. He concluded that " the affinities of *Atoxoplasma* lies not with the toxoplasms but with the haemogregarines, and that failure to achieve transmission by subinoculation is not due to strict host specificity but to the fact that the method employed has simply not resulted in the transfer of stages able to establish infection in another vertebrate ". He therefore proposed to amend the diagnosis of *Atoxoplasma* as follows:—

" Benign to mildly pathogenic parasites, typically sausage-shaped with more or less equally rounded ends; occurring in avian monocytes and lymphocytes, often causing pronounced indentation of the nucleus of the latter; their weak staining reaction and the absence of a well-defined periplast rendering it difficult to differentiate their cytoplasm from that of the host cell; nucleus diffuse, granular and with a tiny karyosome; multiplication by binary fission, few (usually two) division products per host cell; not transmissible by subinoculation ".

Laird (1959) reviewed the literature on the *Atoxoplasma* spp. and *Toxoplasma* spp. of birds, and stated that according to the International Rules of Zoological Nomenclature, *Atoxoplasma paddae* (Aragao 1911) (syn. *Haemogregarina paddae* Aragao 1911) must, therefore, be regarded as the type species of the genus *Atoxoplasma*. Consideration of the investigations makes it apparent that the genus *Atoxoplasma* is distinct from the genus *Toxoplasma* and that the former need not be considered in the generic classification of the *Toxoplasma*, *Besnoitia* and *Fibrocystis* parasites.

The last question that arises from this discussion is the status of the genera *Toxoplasma*, *Besnoitia* and *Fibrocystis* in the higher taxonomic categories. Wenyon (1926), Neveu-Lemaire (1943) and Reichenow (1953) concluded that neither the morphology nor the mode of reproduction of *Toxoplasma* presents any characteristic features, and hence this genus cannot be placed in any natural scheme of classification of the Protozoa. Westphal (1953) expressed the opinion that the genus *Toxoplasma* might be related to the genus *Leishmania* Ross 1903, from which it can be differentiated by the absence of the blepharoplast. In support of this theory he drew attention to *Trypanosoma equinum* Voges 1901, which has lost its blepharoplast, and suggested that the same could have happened with the *Toxoplasma*.

In conclusion, the writer wishes to state that he favours the conservative attitude adopted by Wenyon (1926), Neveu-Lemaire (1943) and Reichenow (1953), and proposes that, for the present, the genera *Besnoitia* and *Fibrocystis* be included with the genus *Toxoplasma* in the group of " Parasites of Doubtful Nature ".

The incomplete classification of these three genera is as follows:—

GENUS: *Toxoplasma* Nicolle & Manceaux 1909.

T. gondii (Nicolle & Manceaux 1909. [*Ctenodactylus gondi* (Rothmann)].

Synonyms: *T. pyrogenes* Castellani 1913. (Man).
T. canis De Mello 1910. (Dog).
T. cuniculi Splendore 1908. (Rabbit).
T. ratti Sangiorgi 1914. (Rat).
T. caviae Carni & Migliano 1916. (Guinea pig).
T. sciuri Coles 1914. (Squirrel).
T. talpae Prowazek 1910. (Mole).

GENUS: *Besnoitia* Henry 1913.

B. besnoiti (Marotel 1913). (Cattle, sheep, goat and rabbits).

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Synonyms: *Sarcocystis besnoiti* Marotel 1912.
Globidium besnoiti (Marotel 1912).
Gastrocystis besnoiti (Marotel 1912).
B. bennetti Babudieri 1931. (Horse).

Synonym: *Globidium besnoiti* (Marotel 1912).
B. jellisoni Frenkel 1953. (White-footed mouse).

GENUS: *Fibrocystis* Hadwen 1922.
F. tarandi Hadwen 1922. (Reindeer).

VII.—TRANSMISSION

A.—Natural transmission

The natural mode of transmission of *B. besnoiti* is obscure. Investigators in this field of research have advanced several theories which comprise (*a*) transmission by contact, (*b*) mechanical transmission and (*c*) biological transmission.

(*a*) *Transmission by contact.*—Bennett (1933) and Hofmeyr (1945) suggested that transmission follows contact between susceptible and affected animals. In an attempt to confirm this assumption, affected cattle were allowed to cohabit with fully susceptible animals over a period of ten years at Onderstepoort. All animals had access to the same food manger and water trough. No transmission resulted. These results permit the conclusion that cohabitation is not followed by transmission.

(*b*) *Mechanical transmission.*—In order to obtain evidence whether this method of transfer is possible, the writer employed rabbits as reservoirs showing a microscopic blood infection of *B. besnoiti*. Transmission could be obtained readily by introducing the tip of a seventeen gauge hypodermic needle into the ear vein of the donor, and immediately after its removal inserting it either into an ear vein or into the skin of several rabbits. This successful transmission indicates the possibility of a mechanical transmission by arthropods during the period of a microscopic blood infection. It must, however, be stressed that the period of an active infection is of short duration in cattle and that mechanical transmission would be a matter of chance. Even if one did accept that this method of transfer does occur, one is still faced with the question of how the first cases are produced at the beginning of the bovine besnoitiosis season, when the only known source of infection is the convalescent cyst carriers. Should such animals serve as reservoirs, then one must expect the vector to be endowed with powerful and deeply penetrating mouth parts, capable of piercing the cyst wall. Comparison between the mouth parts of various blood sucking insects suggests that members of the family Tabanidae may fulfil these requirements. It thus appears that this avenue of approach may be fruitful for determining the mode of transmission.

(*c*) *Biological transmission.*—The seasonal incidence of bovine besnoitiosis caused Barrairon (1938) and Herin (1952) to suggest that this disease may be transmitted by an arthropod. They did, however, not state specifically whether transmission is effected mechanically or biologically. The requirements for the former method have already been discussed. Should the latter method be involved then the vector must also possess well developed mouth parts, and be capable of surviving the interepizootic period. This is not impossible if one considers the seasonal

incidence of sweating sickness, where *Hyalomma truncatum* Koch, with its strongly developed mouth parts, is responsible for the transmission. Although there is no evidence that ticks are involved in the transmission, it is nevertheless suggested that this avenue be explored.

B.—Artificial transmission

(*a*) *Attempts to transmit the disease with cutaneous parasites.*—Besnoit & Robin (1912) tried to transmit *B. besnoiti* to cattle by injecting cyst suspensions through the intracutaneous or subcutaneous route, but without success. The insertion of finely-cut, infected skin strips into the skin of susceptible animals did not result in transmission. Franco & Borges (1916) failed to infect rats and mice by administering cyst suspensions through either the oral or subcutaneous route.

During 1953 the writer repeated this work, using pieces of skin from heavily parasitized cattle. These specimens were suspended in physiological saline and finely mixed in a Waring blender. Examination of the suspensions revealed the presence of numerous motile spores. The inoculum, in the form of an opaque suspension, was administered intravenously into two oxen and subcutaneously into two other recipients, at the rate of 5·0 ml. per 50 Kg. body weight. In another instance a thin sheet of connective tissue, approximately 4 square cm. in size and heavily infected with *Besnoitia* cysts, was implanted subcutaneously into the flank of an ox. The body temperatures of the five recipients were recorded twice daily over a period of two months. Visual inspection was continued for another two months. In not a single instance could any clinical evidence of besnoitiosis be detected. The writer omitted to examine skin sections for the presence of parasitic cysts, and hence it is not known whether or not a subclinical infection had occurred.

(*b*) *Attempts to transmit the disease with blood parasites:—*

(i) *Infection of cattle.*—Failure to reproduce clinical cases of bovine besnoitiosis by means of cyst suspensions, suggested that the work by Cuillé *et al.* (1936, 1937), be repeated by using the blood of actively infected cattle as inoculum. An opportunity arose for such tests during December, 1953, and January and February, 1954, when three head of naturally infected cattle, showing the early stages of the disease, became available. One litre of blood from each of these animals was transfused into three fully susceptible cattle No. 6793, 6839 and 5895. The two donors (No. 6840 and 6851) and the three recipients were observed, and blood and lymphnode smears were examined daily until the primary stage of the disease had passed. Skin biopsies from the dewlap and/or escutcheon were taken at four to five day intervals for a period of two months, and subsequently once a fortnight, until a period of six months had lapsed. The skin specimens were sectioned and stained with Haemalum-eosin.

The results of these observations have been reported upon by Pols (1954 a) and are again detailed in Table B of the Appendix. It will be seen that the three recipients developed besnoitiosis, as judged by eventually positive skin biopsies, even though the infection in one case (No. 6793) was clinically inapparent. In two cases no parasites could be detected in blood and lymphatic gland smears, while in the one (No. 6839) organisms were visible in the blood 11 days after artificial infection i.e. one day after the commencement of fever. Cysts were first observed in skin biopsies 23 to 34 days after infection and 13 to 28 days after the initial rise in temperature.

One further ox to ox transmission was undertaken using 250 ml. blood from an infected ox by the intravenous route. In the recipient (No. 5870) *B. besnoiti* appeared in the blood nine days later, simultaneously with the temperature elevation. No attempt was made to search for cutaneous cysts.

(ii) *Infection of rabbits, mice, rats and guinea pigs.*—Consideration of the course of the disease in artificially infected cattle makes it apparent that this species is not suited for large scale experiments, and that the maintenance of *B. besnoiti* in one or other species of laboratory animal would greatly facilitate investigations. (Pols, 1954 a).

With this object in view, infected blood of a naturally infected ox (No. 6840) was used as inoculum, and injected either intraperitoneally or subcutaneously into six mice (1·0 to 2·0 ml.), four rats and four guinea pigs (2·0 to 3·0 ml.). It was impossible to state whether or not they had developed an inapparent form of the disease. On the other hand, it was determined that rabbits are highly susceptible (Pols, 1954 a). Three rabbits (RH, RB and RT) were infected with blood, using 3·0 ml. intravenously, 5·0 ml. intraperitoneally and 10·0 ml. subcutaneously, respectively from the same donor. Eleven days after infection rabbit RH died very suddenly. Blood smears showed the presence of *B. besnoiti* trophozoites. On the same day swelling of the hind limbs was noted in rabbit RB. The following day the swellings also appeared at the base of the ears. The blood smears were positive. The animal was killed *in extremis*, and a further three rabbits were injected in order to maintain the Fuls *B. besnoiti* strain by serial passage. Observations on the maintenance of the strain are listed in Table C of the Appendix. Rabbit RT had a more prolonged incubation period, which was followed by a more pronounced generalised oedematous swelling, and a large number of parasites in the blood as seen on two consecutive days. The parasites were morphologically identical with those encountered previously in cattle.

In order to fulfil Koch's postulates, 13·0 ml. of blood from the latter rabbit, killed *in extremis*, was injected into an ox (No. 5887). The animal developed fever accompanied by mild clinical symptoms, and 23 days later cysts were seen in skin sections.

Having determined that transmission can be effected readily in rabbits by means of infective blood, attempts were made to establish whether the disease can also be transferred by means of either serous fluid present in the oedematous swelling of the subcutis, or suspensions prepared from liver and spleen of diseased animals. Serous fluid, obtained from the pronounced oedematous swelling at the base of the ears of rabbit No. 13, was injected subcutaneously into a susceptible rabbit. The quantity used was 5·0 ml. The recipient developed a typical reaction accompanied by a microscopic blood infection, and died 11 days later. The intraperitoneal injection of 5·0 ml. of a suspension prepared from the spleen of rabbit No. 1305, into rabbits No. 1308 and 1309 was followed by a typical reaction in both animals. These experiments thus showed that transmission is possible by means of materials other than blood.

Earlier in this dicussion it was stated that, in the case of cattle, cohabitation between diseased and susceptible animals is not followed

by transmission. In order to verify this statement an attempt was made to infect rabbits, using either an infected suspension prepared from the liver and spleen or a mixture of faeces and urine from actively affected rabbits, and administering these materials by the oral route. A stomach tube was used for the administration. Two rabbits (No. 1660 and 1661), each of which had received 10·0 ml. of the spleen and liver suspension, failed to react. On challenging their immunity one month later they proved to be fully susceptible. Two controls (No. 1658 and 1659) that had received 5·0 ml. of the same organ suspension interperitoneally both contracted besnoitiosis and died. Although these tests have shown that infection by the oral route is not followed by transmission, attempts were nevertheless made to infect rabbits by means of excreta from actively infected animals. For this purpose 20·0 ml. of a suspension of faeces in urine was given to two rabbits (No. 1654 and 1657) by the oral route. Both animals were observed for a month without showing a reaction. The experiment was thereupon repeated on another two rabbits (No. 1675 and 1676), each of which received 30·0 ml. of an excreta suspension *per os*. Both failed to react, and on challenging their immunity one month after infection they proved to be fully susceptible.

Consideration of these results makes it apparent that transmission does not result by the oral route even when the organ material used contained active *B. besnoiti* parasites. Cohabitation between actively affected and susceptible animals should, therefore, not be followed by transmission.

C.—Period of infectivity

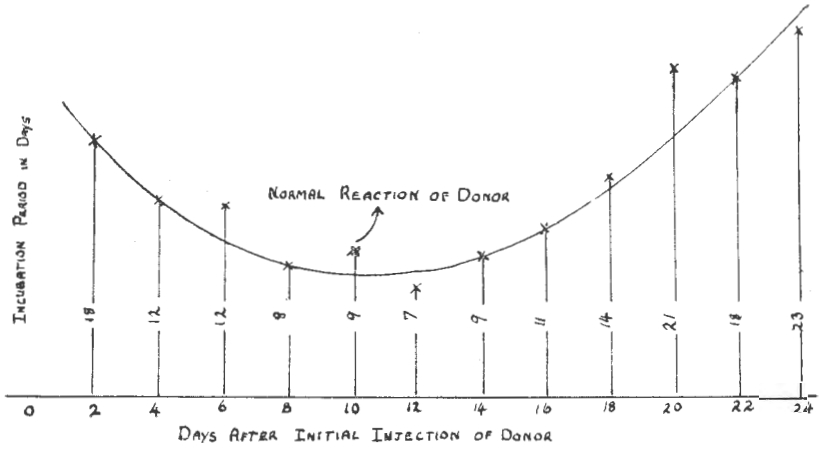
The experiments of Cuillé & Chelle (1936) and the observations recorded previously, indicated that the infection can only be transmitted artificially by means of blood from cattle during the primary stage of the disease. Experimental work was not done on either cattle, sheep or goats to determine critically, by means of repeated subinoculations of blood, the period of infectivity during this stage. One incidental observation may be recorded here, indicative of a very brief infective period. Blood from heifer No. 6839, collected on the day when the peak of the temperature reaction was obtained, proved to be infective for a rabbit. This peak occurred two days after the initial rise of temperature. The subinoculation was repeated five days later. By that time the temperature had subsided to 104° F, and the cutaneous oedematous swelling had just begun to develop. None of the four rabbits used for the second subinoculation showed any reaction. Their immunity was challenged two months later and all proved to be fully susceptible.

As the rabbit had proved a pre-eminently useful laboratory subject, the following experiment was conducted to determine the duration of the period of infectivity of rabbit blood after an artificial infection. Subinoculations were performed from an infected donor by injecting 5·0 ml. of its blood intraperitoneally into one susceptible recipient two days after the date of infection of the donor, and repeating this process every alternate day for 24 days. The donor reacted on the fifth day by elevation of temperature. On the tenth day the peak of fever was reached, and trophozoites could be demonstrated in the blood. In the normal course of events, this would have been the time selected for subinoculation. All 12 recipients reacted thus, indicating that the donor's blood had been infective during the total period under test, i.e. throughout the incubation period, and up to 14 days after the peak of reaction. Another rabbit, which had been injected 47 days previously, was used

as donor 30 days after the first appearance of parasites in the blood. Two susceptible rabbits received 5·0 ml. of blood intraperitoneally. No reaction resulted. The immunity of one of the recipients was challenged two months later and proved to be fully susceptible. The other rabbit died from an unknown cause.

Consideration of these results makes it apparent that the duration of the period of blood infectivity, as determined in a single rabbit (24 days), is considerably longer than that observed in a single heifer. It is, however, impossible to conclude from these tests whether or not the end point had been determined accurately for both species of animals.

An interesting fact emerged from the first-mentioned rabbit experiment. The length of the incubation period in the positively reacting recipients increased proportionately with the time interval elapsing between transmission from and peak of infection of the donor. This may be illustrated graphically as follows:—



Graph illustrating the difference in length of the incubation period.

VIII.—Epizootology

The present state of the knowledge of the epizootology of bovine besnoitiosis is far from complete as the natural mode of transmission is obscure. Observations made in Southern Africa during the last two decades have shown that cyclical variations in the incidence of the disease do occur and that they are similar to those seen in horse-sickness, bluetongue, three day sickness and sweating sickness. General experience has been that a large number of cattle contract besnoitiosis in December, January, February and March, while only sporadic outbreaks occur in autumn, winter and spring. Observations made in other enzootic regions of Africa and Europe have also shown that the highest incidence is in summer. The presence of susceptible cattle determines the degree of prevalence.

In Transvaal, the distribution extends widely into the bushveld and bankenveld areas, with an altitude of up to 1,200 metres above sea level and an average rainfall of 600 mm. per annum. The natural water supply on the infected farms varies considerably. Some farms border on perennial rivers, others are situated along rivulets containing running water only during the summer rainfall season, while the remaining farms depend mainly on a subterranean supply of water or, alternatively, on water collected in dams.

The source and maintenance of bovine besnoitiosis in nature still needs to be determined. This becomes evident when one considers the sporadic outbreaks of equine besnoitiosis. Field observations revealed a single affected horse in the Grahamstown district in the Eastern Cape Province, and ten cases in the Vryheid and Umfolozi districts in Natal (*vide supra*—Distribution, Table 1). The source of infection is mysterious when one considers the fact that neither before nor after the outbreaks had cases of equine besnoitiosis been detected. Consideration of these events suggests that an animal other than a soliped may have served as a reservoir. It thus becomes apparent that a clear conception of the epizootology will only become available when the natural mode of transmission has been determined.

IX.—PATHOGENICITY

A.—Susceptibility to natural infection

Extensive observations by numerous investigators on the incidence of bovine besnoitiosis, listed in Table 1, have shown that this disease has so far only been encountered in cattle. From field surveys conducted in the Transvaal it appears that stock older than 18 months are highly susceptible, while the course of the disease, as observed in a few younger animals, is relatively mild. Attempts to establish the degree of susceptibility of cattle did not entertain much hope when it became apparent that the five artificially infected adult animals listed in Table B of the Appendix developed either mild or inapparent reactions. Consideration of these results indicated that for studies on the pathogenicity of *B. besnoiti* in different age groups, a very large number of animals would have been required, and even then it would have been questionable whether observations on artificially infected cattle were comparable with those described from natural cases.

When an outbreak occurs in a herd only a relatively small number of animals shows clinical symptoms. There is no difference in susceptibility between different breeds and animals of either sex; the indigenous Afrikaner breed is just as susceptible as the exogenous breeds, Hereford and Short Horn. Although the mortality rate does not exceed ten per cent, the disease is nevertheless responsible for severe economic losses. Affected animals lose condition, milk production decreases considerably, cows may abort, bulls often become sterile, and hides are valueless for tanning. (Pols, 1954 a).

The relatively small number of animals that become visibly infected in any one season, even in large herds, gives rise to speculation. Of the theoretical possibilities, the only one at present worthy of serious consideration is that of clinically inapparent infections in very young and possibly also in some of the mature stock, with a consequent state of premunition. The fact that susceptible animals, newly imported into an enzootic region, often contract the disease during the first besnoitiosis season seems to support this theory.

B.—Susceptibility to artificial infection

(1) *Attempts to infect laboratory animals*

(*a*) Actively affected cattle used as source of infection:—

The successful transmission of *B. besnoiti* to rabbits by means of blood derived from ox No. 6840 and heifer No. 6839 has already been described. At the time when the susceptibility of rabbits was established, an attempt was also made to transmit the infectious agent to guinea pigs, rats and mice. As none of these laboratory animals developed a clinical reaction after the intraperitoneal injection of blood from ox No. 6840, it was assumed that they were not susceptible.

(*b*) Actively affected rabbits used as source of infection:—

Inoculation of 2·0 to 3·0 ml. infected rabbit blood either subcutaneously or intraperitoneally into 16 guinea pigs, resulted in temperature reactions of up to 106° F appearing in ten cases. The incubation period varied from four to 17 days. Isolated trophozoites were found in blood smears in two cases eight and 15 days after infection. In a third guinea pig the presence of parasites was determined by a biological test on a rabbit. Several serial passages in guinea pigs were conducted, but these animals developed nothing more than an irregularly intermittent temperature. These tests showed that although the guinea pig is susceptible, it is not suited for laboratory tests.

Further transmission experiments showed that no demonstrable reactions, as judged by pyrexia, symptoms and blood smear examinations were obtained after injecting infective rabbit blood into mice, rats, hamsters, fowls and a rock dassie (*Procavia capensis* Pallas). The observations are listed in the subjoined Table 4.

TABLE 4

Observations on artificially infected laboratory animals

Species	No. Injected	Quantity of Blood in ml.	Route of Infection	Result	Smear Examination
Mice.....	16	1·0 to 2·10	ip. or sc.	N.R.....	Negative.
Rats.....	6	2·0	ip. or sc.	N.R.....	Negative.
Hamsters.....	2	2·0	ip.....	N.R.....	Negative.
Dassies.....	4	4·0	ip.....	N.R.....	Negative.
Pullets and cockerels.....	8	2·0 to 3·0	ip., sc., iv.	N.R.....	Negative.
Adult hens.....	6	3·0 to 5·0	ip., sc., iv.	N.R.....	Negative.
Adult cocks.....	2	3·0 to 5·0	sc., iv.....	N.R.....	Negative.

N.R. = No reaction.

These animals were observed for a period of six weeks. No attempts were made to ascertain whether any of them had developed an inapparent infection. The tests nevertheless showed that these animals are not suited for laboratory tests.

(2) *Attempt to infect sheep, goats, horses and a dog*

Actively affected rabbits used as source of infection:—

(*a*) *Susceptibility of sheep and goats.*—For these experiments three rams, one ewe and four billy goats, which had been raised in a non-enzootic bovine besnoitiosis area, were employed. The observations on these animals are detailed in Table C of the Appendix. Sheep (No. 86569) and goat (No. 79328) each received 350 ml. of rabbit blood by the intravenous route.

After an incubation of seven days the sheep showed pyrexia and anorexia for several days. No parasites could be demonstrated in blood smears, but they appeared in two rabbits used for the biological test. The immunity of this animal was challenged 14 months later. It proved to be solidly immune as no reaction was noted over a period of four months. In contradistinction to the sheep, the goat showed distinct clinical symptoms. The temperature started to rise six days after the artificial infection. Two days later the animal appeared ill and showed swelling of

the nose. After four days the face and anterior portion of the neck became oedematous, eyes showed lachrymation, and a mucoid discharge from the nose appeared. The animal was disinclined to move and anorexia persisted for a week. The temperature rose to a peak of 107° F 15 days after infection. Blood smear examination failed to reveal any parasites. In two rabbits that received 5·0 ml. blood intraperitoneally, *B. besnoiti* could be demonstrated in the blood film. The subcutaneous swellings subsided a fortnight after their onset, and a week later young cysts were found in the skin sections. For three months thereafter a slight diffuse alopecia was noticeable over the anterior half of the body. Skin lesions, so striking in bovine besnoitiosis, never appeared. There was considerable loss in condition. Gradual recovery ensued during the following five months.

Serial passage of *B. besnoiti* in sheep and goats was next attempted. Sheep (No. 89632) and goat (No. 33537) each received 30·0 ml. blood from rabbit No. 1468. Both animals showed no symptoms other than pyrexia. In the goat, cysts could be demonstrated in the skin three-and-a-half months after the artificial infection. In both animals the presence of *B. besnoiti* was determined by biological tests on rabbits. Blood from the sheep and goat was collected at the height of the febrile reaction, and 50·0 ml. was injected intravenously into a sheep (No. 615) and a goat (No. 88549), both of which developed pyrexia after six and four days respectively. Biological tests conducted on rabbits showed that both the sheep and goat harboured *B. besnoiti*. The serial passage was continued for only one further generation in a sheep (No. 145) and in a goat (No. 88536). Again 50·0 ml. blood collected at the height of the temperature reaction was used as inoculum. The recipients showed pyrexia a week later. *B. besnoiti* could not be demonstrated in the daily blood smears of the sheep and goat, but appeared in the rabbits used for the biological test

Consideration of these results makes it apparent that although sheep and goats are susceptible to *B. besnoiti*, the serial passage in these animals did not enhance the virulence of the parasite. Trophozoites could not be demonstrated microscopically in blood smears from these animals even though cutaneous cysts were detected in two of the goats.

(*b*) *Susceptibility of the horse and dog.*—The two horses and the dog used for these tests had been reared in a stable kept under tick-free conditions at Onderstepoort, and had never been exposed to bovine besnoitiosis. The inoculum consisted of a pooled spleen and liver suspension and/or blood. The horses each received 30·0 ml. of the organ suspension and 30·0 ml. blood intravenously, while the dog received 10·0 ml. by the same route. The two rabbits used for the serial passage of the *B. besnoiti* strain, served as controls. Both reacted promptly to the disease. No demonstrable reactions were seen in either the horses or the dog over a period of three months. As no biological tests were conducted on rabbits, it is impossible to state whether or not an inapparent form of the disease had occurred.

Consideration of the studies on the pathogenicity makes it apparent that, besides cattle, susceptibility to *B. besnoiti* has also been established in sheep, goats, rabbits and guinea pigs. Critical experiments, based on biological tests on rabbits, will have to be conducted to determine whether or not mice, rats, hamsters, fowls, dassies, horses and dogs develop infection. It is also recommended that studies on the pathogenicity be extended to include wild animals, as they may disclose unknown reservoirs of *B. besnoiti* serving as a source of infection in nature.

X.—Pathogenesis

The course and pathogenesis in bovine besnoitiosis follows the general pattern of most infectious diseases in as much as there is an acute phase with parasitaemia, fever and general symptoms of malaise, followed by localisation in a particular tissue, and consequently a more chronic phase ensues. It is unique in this respect, that the tissue involved is the more superficially situated connective tissue, i.e. the dermis, subcutaneous tissue, fascia, as well as the connective tissue of the scleral conjunctiva, nasal and laryngeal mucous membranes. More deeply situated connective tissue is very rarely parasitised, and then only to a slight degree. Although clinically complete recovery may follow mild attacks of the disease, one cannot really speak of a complete restitution, as a certain degree of sclerodermia, however slight, undoubtedly persists, as do cysts containing viable organisms.

Mortality, directly attributed to *B. besnoiti* infection only, is low. Few such cases were seen by the author. References to such cases in the literature are not clear enough to allow critical evaluation on this point. If death occurs, it will often be due to early or late complications. Of the former, secondary infection through the severely affected and rapidly developing decubital lesions, as well as the result of deranged physiology of the skin (massive exudation) and possibly the effect of the toxin produced by the parasite, are the most important factors.

In the rabbits, on the contrary, uncomplicated deaths during the acute phase of the disease are fairly frequent. It is considered that the anaemia which is such a prominent symptom in the rabbit, plays an important role in this respect. It should also be borne in mind that blood parasitaemia in the case of the rabbit is much more pronounced.

Cattle surviving the acute phase do not die as a rule, although severe cases waste away and remain unthrifty and, therefore, more subject to late complications. In rabbits more deaths have been experienced, but here intercurrent infections, a condition inherent in captivity, form complicating factors that are impossible to analyse accurately.

Cutaneous manifestations are not clinically apparent in the rabbit after the primary stage of the disease. Compared with the heavy infection in the blood stream, the number of cysts found in the skin is comparatively low, especially when one considers the rarity of parasites in the blood circulation of cattle and the subsequent density of infection often found in the skin.

The ensuing immunity was shown to exist in all cases tested, in cattle from seven months to three years, in sheep from five to 18 months, in one goat after five and 18 months, and in rabbits from 70 to 112 days after date of initial infection. The view has already been advanced that the nature of the immunity is one of premunition or *immunitas non sterilisans*, as cysts with viable organisms could be demonstrated in cattle 10 years after infection, and thus are presumed to remain for life.

The deleterious effect of *B. besnoiti* on spermatogenesis is an interesting and important finding. Its temporary presence forms a useful indication in laboratory experiments, and is of considerable importance to the cattle breeder and clinician. Although exact information on spermatogenesis in most other infectious diseases is lacking, nevertheless it would appear that sperm production is interfered with to a relatively more severe degree in besnoitiosis, when compared with the severity of

clinical symptoms. Except for the occasional cyst in the connective tissue in the testis, these organs are not grossly affected. Circulating parasites are few and far between in the acute phase and absent subsequently. Hence influences other than the direct presence of parasites or fever must affect the testes.

XI.—SYMPTOMATOLOGY

A.—Besnoitiosis in cattle, sheep and goats

The first account of a disease in cattle, referred to as " l'elephantiasis et de l' anasarque du boeuf ", was published by Cadéac (1884). In subsequent publications Besnoit & Robin (1912, 1914) gave a lengthy account of the malady. The description of the symptoms was accompanied by photographs of affected cattle and cutaneous cysts harbouring the causal agent. Further records on the symptomatology in naturally infected convalescent cases were published by Franco & Borges (1916), Bennett (1931), Hofmeyr (1945) and Pols (1954 a), while the course of the disease in artificially produced infections was given by Cuillé, Chelle & Berlureau (1936), Cuillé & Chelle (1937), Barrairon (1938) and Pols (1954b).

During the course of the present investigations observations were made on 50 affected cattle. Of these, three were submitted to Onderstepoort from the field when they showed the febrile phase of the disease, seven were produced artificially, and the remainder were chronically affected natural cases. The five sheep and five goats included in these studies were all artificially produced cases.

Field surveys in the Northern Transvaal by the investigators, listed in Table 1, have shown that severe cases, exhibiting the full range of the symptoms, occur relatively infrequently. The writer only observed one very severe case (bull No. 6851, Table B of the Appendix) during the whole period of illness which terminated fatally. From observations on experimental cases in cattle (Tables B and G of the Appendix) one may conclude that the incubation period varies from six to ten days, and that pyrexia persists for three to six days. As a rule the temperature fluctuated between 103° F to 107° F. Anorexia, depression and listlessness constituted accompanying signs. Photophobia was usually a distinct symptom, as was the hot, painful, subcutaneous, oedematous swelling affecting any part of the body, but with a predilection for one or more limbs and/or pendulous regions (dewlap, scrotum and flank) at, or shortly after, the peak of the febrile reaction. Except in severe cases, the swelling, although in itself diffuse, remained restricted to a particular region or regions. The swollen area became indurated as the disease progressed. The enlargement of lymphnodes, which was a common symptom in the primary stage of the disease and of diagnostic value, was present to a variable degree. In experimentally produced cases, however, it was moderate or absent. That cases do occur with virtually no fever, and no anasarca or any other symptoms commonly seen in the primary stage, was exemplified by cow No. 6793 (Table B of the Appendix). The extent and severity of the skin lesions in the second and third stages of the disease varied widely, more or less proportionally to the preceding swelling. The reactions in the four experimentally produced cases (No. 6839, 5895, 5887 and 5870, Table B of the Appendix) were mild. In two cases only pyrexia was seen, and in the remaining two animals fever was accompanied by subcutaneous oedematous swellings of the limbs. At the height of fever the animals were listless and showed inappetence. Permanent skin lesions were not prominent.

Regarding the course of the disease, it may be stated that in severe cases the secondary depilatory stage is the critical phase on which the prognosis is based. The advanced skin lesions, with folding and cracking and serous-sanguinous exudate, form a favourable port of entry for secondary bacterial infections, which is the factor often responsible for death. The clinical syndrome then becomes one of a bacteraemia.

Animals surviving this phase usually do not die, but progress to the third or seborrhoea sicca stage (Hofmeyr, 1945). The severely affected cases remain emaciated for months on end. For all practical purposes such animals are poor doers, and represent a total economic loss. Although one may speak of recovery in the sense that active inflammatory processes are no longer present, the skin remains thickened and wrinkled for life. A certain amount of hairgrowth will again occur. In such skin the cysts are found studding the cutis vera, and are present to a lesser extent in the subcutis, superficial fascia, without evidence of any inflammatory reaction. A very slight skin infection produces no clinically visible skin pathology. The chances of encountering cysts in skin biopsies of such cases would be rare indeed.

Cysts, which appeared structurally normal and harbouring viable parasites, remained unaltered at the sites mentioned for the rest of the animal's life. Ten years after the original infection the author still found cysts. It is in this sense that the statement: " recovered animals remain carriers for life " was made by Pols (1954 a). There was no evidence that the cysts ruptured to liberate their contents on the surface of the skin or discharged them into the host's blood circulation.

The impression was gained that the pendulous parts and loose folds of the skin formed predilection sites for the development of pathological changes, namely in the flank fold, scrotum, dewlap and eyelids, thus corresponding to the areas which were swollen during the primary stage of the disease. The only exception to this correlation was found in the limbs, where clinically visible changes generally were by no means as striking as the preceding swellings. No systematic investigations on the differential distribution of cysts over the whole cutaneous area were undertaken.

Clinical observations which had shown that *B. besnoiti* cysts often appeared in the sclera of naturally affected cattle (Hofmeyr, 1945), raised the question of whether this phenomenon could be used as a reliable method for making a diagnosis. For the evaluation of this symptom as a diagnostic method, observations were made on 15 natural cases of typical chronic bovine besnoitiosis at the Municipal Abattoir of Pretoria. In nine of these animals distinct white nodules could be seen macroscopically and microscopically in the sclera and skin sections; four animals showed indistinct nodules not of parasitic origin, as determined microscopically; and in two cases no globules were demonstrable even though skin sections revealed the characteristic parasitic cysts. In mild natural, as well as in experimentally produced cases, they were frequently not apparent upon visual examination.

At autopsy no internal pathological lesions directly referable to besnoitiosis were observed, with the exception of cachexia and the presence of cysts, visible as opaque, whitish, raised nodules (0·5 mm. in diameter) in the mucous membrane of the upper respiratory tract and that of the trachea, up to its bifurcation into the bronchi, and in the cutis, subcutis and aponeuroses of the muscles. No cysts were ever encountered in mucous membrane of either the abomasum, or small and large intestine. An occasional cyst was found in the capsule of the spleen, in the connective tissue around the pampiniform plexus and in the tunica albuginia of the testis.

Clinical symptoms exhibited by the four artificially infected sheep did not present any characteristic features. For a few days pyrexia and anorexia were the only observable symptoms. Fever persisted for six days, rising steeply to a peak of about 107° F and then subsiding gradually to normal. The only pregnant sheep of this group aborted eight weeks after the height of reaction. No parasites could be demonstrated in blood smears of any of the sheep nor could cysts be found in skin sections. The presence of *B. besnoiti* infection could only be proved by subinoculation into rabbits at the peak of the febrile reaction, and by subsequent immunity to challenge.

In the four experimentally infected goats the temperature was essentially similar to that in sheep. They appeared visibly ill and were listless and dejected. As described in detail previously (page 302), one goat (No. 79328) reacted severely with oedematous swelling of the face and neck, lachrymation, mucoid nasal discharge and dyspnoea. This was the only small ruminant that showed cutaneous lesions. No parasites were found in blood smears but cysts were demonstrable in skin sections of two goats.

Economically one of the most important symptoms, namely sterility, must be singled out for special consideration. It has been observed that bulls naturally infected with *B. besnoiti* developed a partial or complete sterility. It was often permanent. Out of nine purebred Hereford bulls sent to this Institute from the Northern Transvaal seven years previously, on account of besnoitiosis and sterility, two (No. 4197 and 4201) were available for the study on the sperm picture.

Bull No. 4201 ejaculated 8·0 ml. of a cloudy fluid in which no sperm was present. On one occasion bull No. 4197 ejaculated 8·0 ml. of milky semen. Sperm motility was good, but there was a large number of loose heads. Ten months later 7·0 ml. of a milky semen was obtained, and although the motility was good, only 35 per cent of the sperms were alive, 12 per cent had loose heads and 8 per cent coiled tails. The fertility of this bull was thus permanently impaired.

Studies on the sterility in besnoitiosis were extended to include two bulls (No. 5981 and 5873), one ram (No. 1952) and one billy goat (No. 3171), which were infected artificially by using infective rabbit blood as inoculum (Table G of the Appendix). In all instances semen samples were obtained by electrical ejaculations before and after infection.

Before infection it was determined microscopically that the sperm picture of the two bulls was almost identical. A count of 65 per cent (bull No. 5981) and that of 68 per cent (bull No. 5873) was recorded. Sperm motility in both cases was good. Semen samples were taken subsequently at approximately weekly intervals for the first eight weeks, and thereafter at roughly monthly intervals, until about seven months had elapsed since the date of infection. Three days after artificial infection the sperm picture was not materially altered. A week to a fortnight later no sperm was present. This picture was only modified by the appearance of some dead sperms at occasional intervals until after 137 days, when bull No. 5981 had 27 per cent live sperms together with all types of abnormal forms in the semen. A month later considerable improvement had occurred, and by the 202nd day the normal sperm picture was restored. Bull No. 5873 only began showing 12 per cent live sperms on the 173rd day. The last examination was done on the 227th day, when 55 per cent live sperms with fair motility were present.

It must be stated that, three weeks after infection, bull No. 5981 developed a Corynebacterium abscess in the left testicle, which became fibrotic. Despite the adverse systemic reaction due to this infection, good recovery of the sperm picture eventually took place on the 202nd day after infection. The other bull (No. 5873) suffered from an intercurrent *Trichomonas foetus* (Riedmüller, 1928), infection 42 days after the artificial *B. besnoiti* transmission. It is considered that the former infection did not modify the effect of the latter on the sperm picture in any material way. The immunity of this bull was challenged with a second injection of infected rabbit blood. The injection was followed by severe shock but no further reaction occurred. The sperm picture was not influenced in any way, and during the ensuing 11 weeks *T. foetus* was not detected.

In the case of the ram and billy goat it was determined that before infection the sperm picture was normal. A count of 80 per cent (ram No. 1952), and that of 76 per cent live sperms (goat No. 3171) was recorded. After infection, semen samples of the former animal were examined 11 times over a period of four-and-a-half months, and those of the latter eight times over a period of three-and-a-half months. The sequence of events followed the same pattern in the small ruminants as in the bulls, but took place within almost half the span of time. Within a fortnight the excellent sperm picture obtained before infection had deteriorated markedly, and within four weeks no sperms, or at least no live ones, could be seen. Approximately two-and-a-half months later, live sperms again appeared in the ram (30 per cent) and goat (49 per cent). Complete recovery was observed within four-and-a-half months in the ram, and within three-and-a-half months in the goat. Both animals did not react when their immunity was challenged one month after recovery. Their sperm picture was not affected.

B.—Besnoitiosis in rabbits

In rabbits, clinical symptoms during the febrile stage were more prominent than in either cattle, sheep or goats under experimental conditions. A well-defined thermal reaction developed in nearly all rabbits. The fever persisted for three to seven days and ranged between 103° F to 107° F, mostly between 105° F and 106·6° F. During the fever animals became listless, refused to feed and lost weight rapidly. At about the peak of reaction, hot, painful, subcutaneous oedematous swellings, especially of the ears, head, limbs and scrotum, developed in the majority of cases. In some males the swelling progressed to a complete necrosis of scrotum and testes. Occasionally the swellings extended over the whole body. A few does developed an oedematous swelling and hyperaemia of the vulva. These inflammatory oedemas lasted from three to 14 days.

In contra-distinction to cattle, the skin lesions did not progress. The swelling subsided, and neither proliferative thickening of the skin, serous-sanguinous exudate nor the seborrhoea sicca stage was ever observed. This may have been due to the fact that mortality in rabbits is so much higher, and only those with relatively light symptoms survived.

A leucocytosis accompanied by anaemia was a characteristic symptom (Pols, 1954 b). Differential counts revealed from 20 to 70 per cent monocytes. The rise in the monocyte count occurred concurrently with the rise in temperature. Even from ordinary blood smears the monocytosis often could be inferred readily. Similarly, the monocytosis declined more or less with the drop in temperature.

At about the peak of reaction, anaemia became apparent in blood smears. Hypochromatic macrocytes were the first to appear. Subsequently polychromasia, commonly seen in normal rabbit blood, became more pronounced, and often punctate basophilia developed. In many cases there was as much as a 50 per cent, or even a 70 per cent, drop in the number of erythrocytes. To illustrate the variation in the blood picture, an artificially infected rabbit, No. 330, with an incubation period of 11 days, two days later showed an erythrocyte count of 1·4 million, and a leucocyte count of 4,400 per c. mm. After another four days the count changed to 1·22 million and 14,300 respectively. A second artificially infected rabbit, No. 321, had an incubation period of eight days. Five days later this animal had an erythrocyte count of 2·64 million and a leucocyte count of 7,800 per c. mm. After four days the figures changed to 3·14 million and 5,200 respectively. At autopsy it was observed that the emaciation in both animals was associated with anaemia and ascites.

The duration of the incubation period varied from three to 15 days, with an average of seven days. In fatal cases the duration of the disease varied from two to 16 days, with an average of six days. In one instance death occurred 38 days after the commencement of fever.

During the early stages of the serial passages of *B. besnoiti* it was noticed that the symptoms were more pronounced than at higher levels of passage. This was illustrated by comparing the observations made on 22 rabbits, used for generations No. 20 to 25, with those on animals employed for generations No. 120 to 125. In the first group two rabbits died from unknown causes, seven were killed for the collection of infective blood, ten died from besnoitiosis, and three recovered. In the second group one rabbit died from shock immediately after infection, seven were killed for the collection of infective blood, and ten recovered completely. Depending upon the severity of the reactions, survivors which had suffered severely took anything from one to three weeks to recover. Generally speaking less severe reactions, and consequently a more rapid recovery, were seen more often in rabbits at higher levels of the serial passages Of all rabbits used, 15 per cent did not show a parasitaemia, even though they developed either fever and/or a moderate oedema of the subcutaneous tissues Over the last five generations the percentage rose to 40 per cent. These observations thus support the opinion, expressed by Pols (1954 b), that the disease took a more protracted and less severe course as serial passages progressed.

Although it was established that the genitalia became affected, no work was conducted to evaluate the effect of *B. besnoiti* on the reproductive ability of both sexes of rabbits. It may be mentioned in passing that a doe (No. 108) gave birth to a litter of seven rabbits two months after the date of infection and seven weeks after the commencement of the disease. The whole litter died within 24 hours. Examination of blood and organ smears, as well as biological tests on rabbits, failed to reveal *B. besnoiti* in the offspring. Since conception had taken place a fortnight after subsidence of the clinical symptoms, and as no parasites were seen in the offspring, no conclusions could be drawn whether or not the deaths were a sequel to besnoitiosis.

At autopsy, rabbits dying from besnoitiosis within a few days after commencement of symptoms, only revealed subcutaneous oedema, ascites and sometimes subepicardial petechiae. Those surviving for longer periods showed, in addition, emaciation and anaemia. Apart from occasional lesions due to intercurrent infections, such as pneumonia, coccidioses, and non-specific enteritis, pathological

changes, not directly referable to besnoitiosis, were seen from time to time. These included either hydrothorax and/or a considerably enlarged gall bladder. The mucous membrane of the stomach and intestine never contained parasites. Macroscopically no nodules, as seen in cattle, could be detected in the cutis, subcutis, fascia, sclera, and nasal, pharyngeal, laryngeal and tracheal mucous membranes.

XII.—Diagnosis

Although a tentative diagnosis of bovine besnoitiosis can be made by considering the epizootology, the clinical symptoms and the lesion at autopsy, definite diagnosis depends upon demonstrating *B. besnoiti* microscopically in either blood or tissue smears, and in sections prepared from either the cutis, subcutis, sclera or mucous membrane of the upper respiratory tract.

Epizootologically, bovine besnoitiosis is restricted to certain regions. When investigations are being made it is essential to determine the local incidence of this malady and other diseases likely to be confused therewith, and the origin of the affected animals. These considerations should guide the investigational procedure.

During the commencement of the thermal reaction of besnoitiosis, a definite diagnosis may present difficulties, as no specific symptoms may be evident. During this period microscopic examination of blood and lymphnode smears may reveal the infectious agent. Failing this, a biological test on rabbits, using blood from the suspect, should be undertaken. Although this is rather a lengthy procedure, it is the only reliable method of diagnosing besnoitiosis during the primary stage of the disease. When applying this test due consideration should be paid to the viability period of the causal agent *in vitro* [*vide supra*—Aetiology, Subsection (*f*)].

As the disease progresses the possibility of making a diagnosis improves. Careful examination of sections prepared from skin biopsies usually reveals *Besnoitia* cysts at different stages of development.

When the patient enters the clinically recognizable phase of the disease no difficulty should be experienced in making a diagnosis. Visual examination will reveal cysts in nasal mucous membrane, and in many instances also in the sclera. Spores can be demonstrated in deep skin scrapings, and in parasitized cysts in sections prepared from skin biopsies.

XIII.—Treatment

As far as could be ascertained from the literature, only a few attempts have been made to treat bovine besnoitiosis. Leitao (1948) employed three injections of 30·0 ml. of a 10 per cent solution of formalin at three daily intervals, for the treatment of chronically affected mature cattle. Herin (1952) advocated a single intravenous injection of 30·0 ml. of a 1 per cent colution of formalin during the primary stage of the disease. For chronically affected adult animals he recommended intravenous administration of 20·0 to 40·0 ml. of Lugol's iodine at four to seven daily intervals. Five injections were usually sufficient. Although no critical evaluation of the results were given, both investigators expressed the opinion that these forms of treatment had a beneficial influence on the course of the disease.

The procedures instituted during these studies involved the use of protozoacidal and bactericidal drugs. Observations on the artificially infected and treated rabbits and the chemotherapeutic agents employed, are detailed in Tables H and I of the Appendix and are summarized in the subjoined Tables 5 and 6 respectively in the text. Observations on rabbits comprised recording the body temperature twice daily, examining blood smears daily during the period of the thermal reaction, and noting the appearance of the subcutaneous oedema of the ears, limbs and external genital organs. The route of administration, the dose per Kg. body weight, the number of treatments given, and the termination of the disease are also listed in the appended tables.

Two series of experiments were conducted. In the first group 53 rabbits of the lower passage levels during 1954, and three of the higher passage levels during 1956 (pentamidine treated rabbits), were treated once or several times during the period of the febrile reaction. The second group, comprising 26 rabbits of the higher passage levels, during 1956 and 1957, were treated during the incubation period, and in 19 of them treatment was continued for either one or more days during the reaction period.

(a) Treatment during the period of reaction

It will be seen from Table H that all 56 rabbits showed a febrile reaction. The incubation period varied from 5 to 15 days. The duration of the disease, which varied from three to 15 days, was, with the exception of one rabbit (No. 96—52 days), within the usual limits seen in untreated animals (*vide infra*—Table C of the Appendix). A microscopic infection of *B. besnoiti* was observed over a period of one, two, three or four days in 50 rabbits. A subcutaneous oedema developed in 41 animals.

In assessing the value of the chemotherapeutic agents, two factors, namely a possible variation in either the morphology or virulence of the parasite, were taken into account. No morphological changes in the parasites suggestive of a specific action by any of the drugs, was observed. If the recovery rate is accepted as a criterion for the specific action of the drugs used, then only the effects of sulfamerazine and pentamidine need be considered.

A total of 19 rabbits was treated with sulfamerazine, and of these six survived (26·3 per cent). Four received a total of 400 mgm. (one recovery), two 800 mgm., one 900 mgm., four 1,200 mgm. (one recovery), one 1,500 mgm., three 1,600 mgm. (one recovery) and four 2,000 mgm. (three recoveries). Should sulfamerazine have been responsible for the recoveries, then one would have expected that, with the increase of the dosage, the recovery rate should have increased accordingly. Although it appears that this has been the case, a definite conclusion on the efficacy of sulfamerazine cannot be drawn, as the number of rabbits employed for dosages lower than 2,000 mgm. was inadequate. The three rabbits used for screening pentamidine as a cure, were derived from the higher *B. besnoiti* passage levels when the virulence of this parasite had decreased for the rabbit. Although two out of the three treated rabbits survived, the recovery is attributed to the decrease in the virulence of the parasite rather than to the specific action of pentamidine.

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TABLE 5

Treatment during the period of reaction

Drug	Number Treated	Severity of Disease	Number that Died
Achromycin and Phenergan	6	1 (+) 5 (++)	1 4
Aureomycin	4	2 (++) 2 (+++)	2 2
Babesin	2	1 (++) 1 (+++)	1 1
Gonacrin	2	2 (+++)	2
Pentamidine	3	3 (++)	1
Phenamidine	5	2 (+) 3 (+++)	0 3
Plasmoquin	1	1 (++)	1
Sodium Iodide	2	1 (++) 1 (+++)	1 1
Sulfamerazine	15	2 (+) 12 (++) 1 (+++)	0 8 1
Sulfamerazine and Phenergan	4	4 (++)	4
Sulfamezathine	12	2 (+) 9 (++) 1 (+++)	2 7 1

\+ = mild
++ = severe
+++ = very severe.

(*b*) *Treatment during the incubation period and in a large number of cases, also during the period of reaction*

It will be seen from Table 1 that all 26 rabbits showed a febrile reaction. The incubation period varied from five to 16 days. The duration of the disease (five to 11 days) was within the usual limits seen in untreated animals of the higher passage levels. A microscopic infection of *B. besnoiti* was observed over a period of either one or two days in 21 rabbits. A subcutaneous oedema appeared in 12 animals.

In assessing the action of the various drugs, several facts became apparent. The duration of the incubation period was not prolonged unduly. No morphological changes in the parasites, suggestive of a specific action of any of the drugs used, was seen. The degree and duration of the microscopic parasitaemia were less than those observed in the previous group of rabbits. The overall mortality rate (36 per cent) was much lower than in the previous group (76·7 per cent). This reduction is ascribed to the decrease in the virulence of *B. besnoiti* following the serial passages in rabbits (*vide supra*—Symptomatology, page 309), and not to the

action of any of the drugs used. Rabbits, with three exceptions, used for the former experiment (Table 5), were derived from the low passage levels during 1954. Animals used for this study (Table 6) were obtained from the higher passage levels during 1956 and 1957. It thus became apparent that the relatively low mortality rate was not due to the specific action of any of the chemotherapeutic agents but were the result of the gradual decrease in the virulence of *B. besnoiti*, following repeated serial passage in a foreign host, the rabbit.

TABLE 6

Treatment during the incubation period, and in a large number of cases also during the period of reaction.

Drug	Number Treated	Severity of Disease	Number that Died
Anthiomaline	1	1 (++)	1
Aralen	2	2 (++)	1
Formalin	4	4 (++)	0
Mixtamycin	1	1 (++)	1
Mixtamycin and Phenergan	1	1 (++)	1
Mycostatin and Phenergan	1	1 (++)	1
Pamaquin	1	1 (++)	0
Phenamidine	1	1 (++)	1
Pentamidine	2	2 (++)	0
Quinacrine	3	3 (++)	1
Spirotrypan	4	1 (+) 2 (++) 1 (+++)	0 1 1
Terramycin	2	1 (+) 1 (++)	0 1
Trypan blue	1	1 (++)	0
Tubomel (INH) and P.A.S.*	2	2 (++)	0

* Para-amino-salicylic-acid.

In conclusion, it can be stated that the apparent cures should be considered with full knowledge that spontaneous recovery in rabbits suffering from besnoitiosis is common. Consideration of these results permits the conclusion that no really satisfactory chemotherapeutic treatment has yet been found, unless the curative effects of sulfamerazine should be fully confirmed.

The observations on the chemotherapy, and the criteria used for assessing the value of drugs as cures, are presented with the hope that they will serve as guides for future investigations.

XIV.—Prophylaxis

Consideration of the gradual dissemination of bovine besnoitiosis into the potential enzootic regions in the Northern Transvaal, and the sporadic outbreaks of equine besnoitiosis within the same regions, as well as in the Eastern Cape Province and Zululand (*vide supra*—Epizootology), makes it apparent that effective prophylactic measures for the prevention of the spread are dependent upon determining the natural mode of transmission and the host range of *B. bensoiti* in nature. Since the cyclical incidence during the summer months suggests that an arthropod may be involved in the transmission, the use of reliable arthropodicides seems to be a feasible control measure. As dipping tanks and spray races are available for the control of tick-borne diseases on many farms, the proposed prophylactic measure can, therefore, be conducted without extra costs. It may also be of value to eliminate chronically affected cattle as they may serve as reservoirs for the infection of vectors.

The development of a reliable method of immunization needs serious consideration, as this will overcome defects of other prophylactic measures. The ideal method of immunization will involve the artificial infection of cattle and controlling the ensuing reaction with a specific drug. Its application is dependent upon the availability of an adequate source of the infectious agent and a reliable chemotherapeutic agent. The former requirement can be fulfilled by using artificially infected rabbits as reservoirs, while the second one still needs to be determined.

Although experiments have shown that artificial infection of mature bulls is followed by a relatively mild form of besnoitiosis, observations on spermatogenesis have revealed that a temporary sterility may persist for periods of up to six months. Recognition of this complication makes it apparent that a specific remedy is urgently needed.

Another approach to the immunization is the artificial infection of very young stock. As severe reactions following a natural infection have not yet been observed in this age group, it is believed that equally mild reactions will follow an artificial infection.

XV.—Immunity

Studies on the nature and duration of the immunity in bovine besnoitiosis were based on observations made on cattle that survived a natural infection, and on cattle, sheep, goats and rabbits which recovered from an artificial infection. Criteria used for assessing the immunity were founded on the persistence of parasitic cysts after recovery, and the clinical and spermatogenic response to challenge.

No reference to immunity could be found in the literature other than that of Hofmeyr (1945), who stated that farmers in the Rustenburg district (Transvaal) had never seen a second attack of besnoitiosis in cattle. The reason for the solid immunity became evident subsequently when it was found that recovered cattle remain carriers of parasitic cysts for life (*vide supra*—Pathogenicity). It may thus be concluded that naturally recovered cattle develop a premunity.

A few observations on the nature and duration of immunity following recovery from an artificial infection, were conducted on an ox No. 5870 (Table B), a bull No. 5873, two billy goats No. 1952 and 3171 (Table G) and a ram No. 86569 (Table D of the Appendix). The immunity of ox No. 5870 was challenged seven months after infection, that of bull No. 5873 after 13 and 36 months, that of goat No. 1952 after six and 19 months, that of goat No. 3171 after four and 18 months, and that

of ram No. 86569 14 months after infection. In the case of the bull and the two billy goats, attention was paid to the spermatogenesis after the first immunity test. Careful observations failed to reveal any clinical symptoms and indications of aspermatogenesis.

The immunity of 18 rabbits was challenged at various intervals of up to 112 days after recovery from an artificial infection. A thermal reaction which persisted for three days was observed in one rabbit two days after challenge. Examination of blood smears failed to reveal any parasites. The remaining rabbits remained normal in every respect. Since sections prepared from skin biopsies, taken at the time when the immunity tests were applied, revealed *B. besnoiti* cysts, it is concluded that the nature of the immunity in rabbits is the same as in cattle, namely an *immunitas non sterilisans*. It thus becomes apparent that the proposed method of immunization can be relied upon to impart a durable immunity.

XVI.—Summary and Conclusions

(1) A brief historical review of bovine besnoitiosis is given.

(2) The importance of the disease as a veterinary and economic problem is stressed.

(3) The identification of the causal agent, *Besnoitia besnoiti* (Marotel, 1913), has made it possible to give a concise definition of the disease.

(4) The synonyms of bovine besnoitiosis are listed.

(5) The distribution of bovine and equine besnoitiosis in Europe and Africa, and the occurrence of murine besnoitiosis in the United States of America are recorded.

(6) A detailed account of the aetiology, based on observations made on rabbits and cattle, is offered.

(7) Systematic studies on the development of the parasite cyst revealed that the cyst wall is of somatic origin.

(8) The inner and intermediate membranes of the wall are derived from the markedly hypertrophied polynucleated host cell, the histiocyte, while the outer wall is formed by collagenous fibres.

(9) The term pseudocyst, as applied by Jacobs (1956) for the *Toxoplasma* cyst where the cyst wall is also of somatic origin, should be used in the same sense for the *Besnoitia* cyst.

(10) Certain chemical components of the parasite and cystic wall have been determined by histochemical tissue reactions.

(11) Asexual reproduction by longitudinal binary fission within either monocytes or histiocytes is the only form of multiplication in the vertebrate host.

(12) The " Fuls " *B. besnoiti* strain has been maintained by serial passage in rabbits for 125 generations. A gradual decrease in virulence of the parasite was noticed as the passage level increased.

(13) *B. besnoiti* remains viable in citrated blood for 48 hours at 20° C, and for 96 hours at + 4° C.

(14) A detailed survey of the complicated history of the nomenclature of certain Protozoa included in the genera *Balbiania*, *Besnoitia*, *Eimeria*, *Fibrocystis*, *Gastrocystis*, *Globidium*, *Haplogastrocystis*, *Ileocystis*, *Lymphocystis*, *Sarcocystis* and *Toxoplasma* is given.

(15) Evidence has been brought forward that *Besnoitia* is a valid genus, that it is related to the genera *Fibrocystis* and *Toxoplasma*, but distinct from any of the remaining genera.

(16) Absence of any characteristic features does not permit placing the genera *Toxoplasma*, *Besnoitia* and *Fibrocystis* in any natural scheme of classification in the Protozoa, and hence it is proposed to group them as " Parasites of Doubtful Nature ".

(17) It has been determined that, besides cattle and rabbits, sheep, goats and guinea pigs are susceptible, while two horses, a dog and several mice, rats, fowls and dassies failed to develop a microscopic infection and clinical symptoms after artificial infection.

(18) The pathogenesis is discussed.

(19) The natural mode of transmission is obscure.

(20) Information on the epizootology is limited.

(21) The symptomatology of bovine besnoitiosis in cattle, sheep, goats, rabbits and guinea pigs is described.

(22) Bulls that survive a natural infection develop either a temporary or permanent sterility.

(23) A temporary sterility, as determined by systematic microscopic semen examination of artificially infected animals, persisted for approximately six months in two bulls, for about three months in a billy goat, and four months in a ram.

(24) Methods for making a diagnosis are described. In inapparent or atypical infections in male ruminants, aspermatogenesis can be used as a guide for making a diagnosis.

(25) Investigations on bovine besnoitiosis have not advanced far enough to evolve reliable prophylactic measures.

(26) Animals that survive either a natural or an artificial infection develop a durable premunity.

(27) Photographs showing the course of the disease in a bull, the pronounced sclerodermatitis in a chronically affected animal, the development of a parasitic cyst in a bull and a rabbit, and the morphology and method of reproduction of *B. besnoiti* are presented.

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A.—The course of severe bovine besnoitiosis in a naturally infected bull No. 6851 (Fig. 1–6).

FIG. 1.—Clinical symptoms during the first phase of the disease. Note the moderate oedema of the limbs.

FIG. 2.—Hindview of bull on same day.

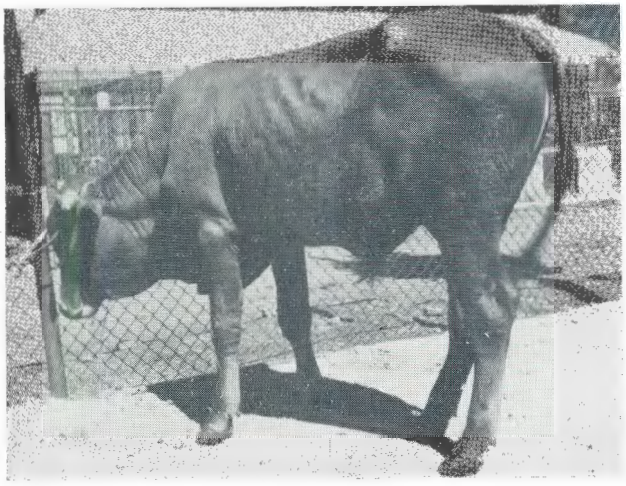

FIG. 3.—Clinical symptoms during the second phase of the disease, thirteen days later. Note decubitus wound on hip and well defined thick skin folds.

FIG. 4.—Hindview of bull on same day.

FIG. 5.—Clinical symptoms during third phase of the disease, 35 days later. Note pronounced emaciation, markedly thickened skin folds and circumscribed areas of alopecia.

FIG. 6.—Hindview of bull on same day.

B.—Advanced case of chronic bovine besnoitiosis in a naturally infected cow. No. 6547 (Fig. 7 and 8).

FIG. 7.—Clinical symptoms four months after infection. Note emaciation, extensive alopecia and indurated wrinkly skin.

FIG. 8.—Head of cow on same day. Note dry and crusty nasal discharge and alopecia.

C.—Typical case of bovine besnoitiosis in an artificially infected rabbit No. 89 (Fig. 9 and 10)

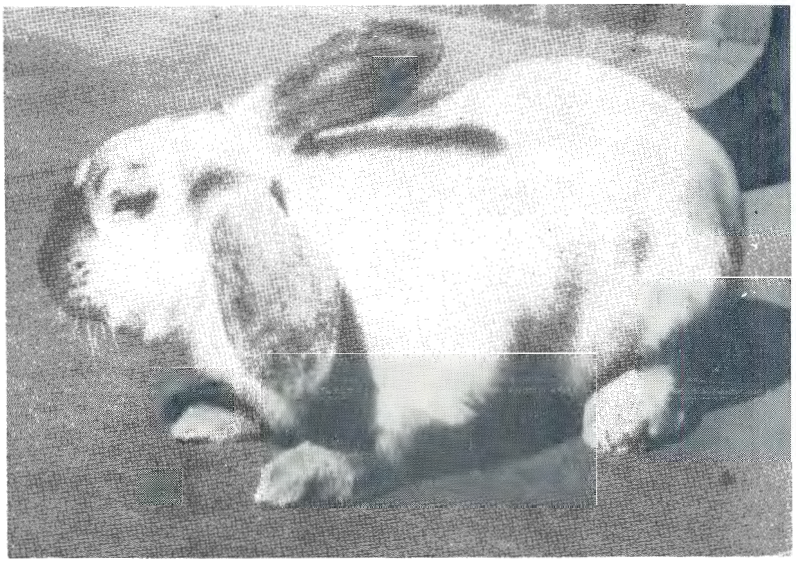

FIG. 9.—Pronounced oedematous swelling of ears, particularly the left one.

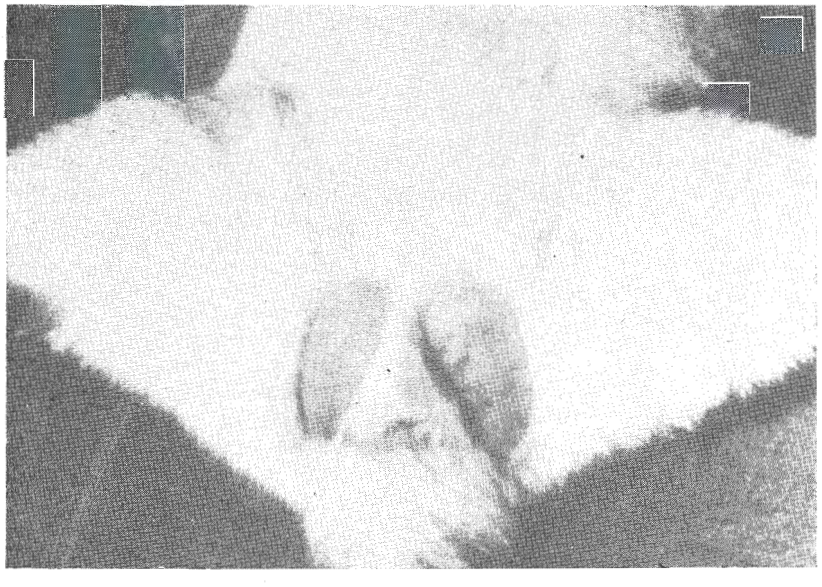

FIG. 10.—Same rabbit, showing oedematous swelling of scrotum.

D.—Skin sections of bull No. 6851 showing the development of *B. besnoiti* cysts over a period of five weeks (Fig. 11–16).

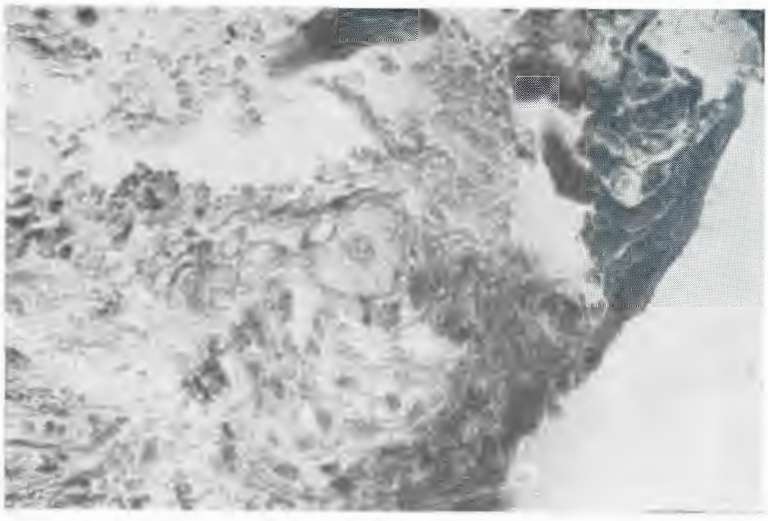

FIG. 11.—Enlarged uninuclear histiocyte surrounded by connective tissue fibres four days after height of thermal reaction × 480.

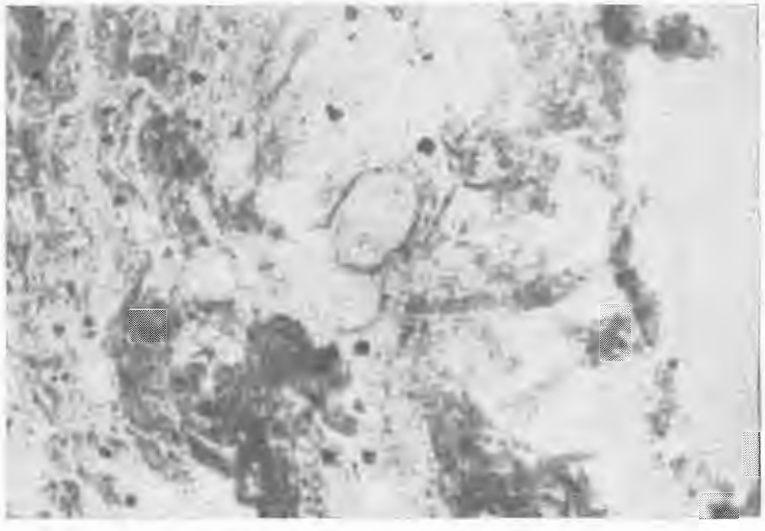

FIG. 12.—Enlarged uninuclear histiocyte containing two *B. besnoiti* parasites on the 7th day × 480.

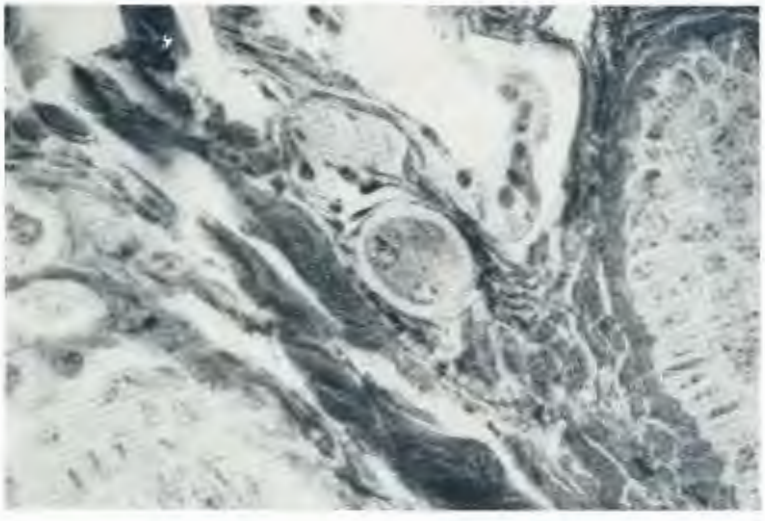

FIG. 13.—Enlarged binucleated histiocyte containing four parasites on eighth day × 480.

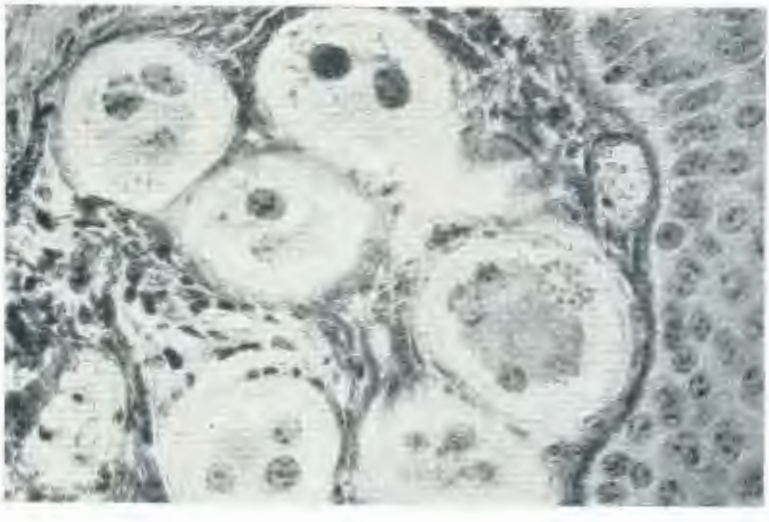

FIG. 14.—Group of multinucleated histiocytes containing vacuoles with parasites and surrounded by connective tissue fibres on 13th day × 480.

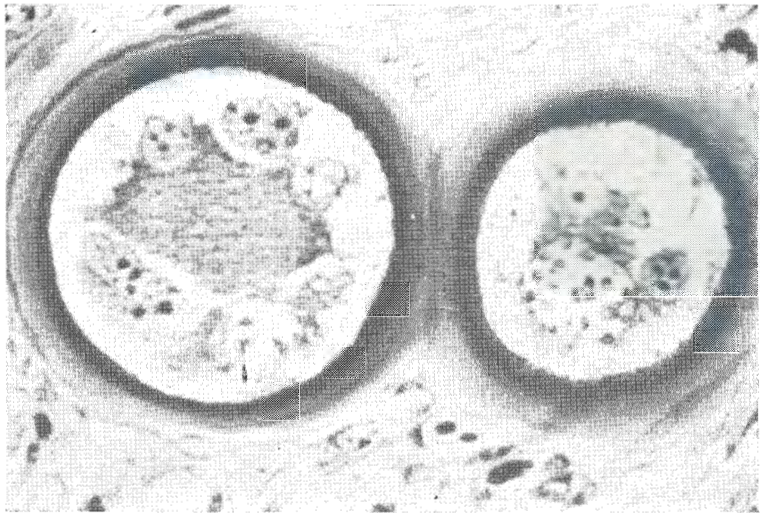

FIG. 15.—Young cysts with well-defined walls, each containing several host cell nuclei, and vacuoles harbouring parasites on 24th day × 480.

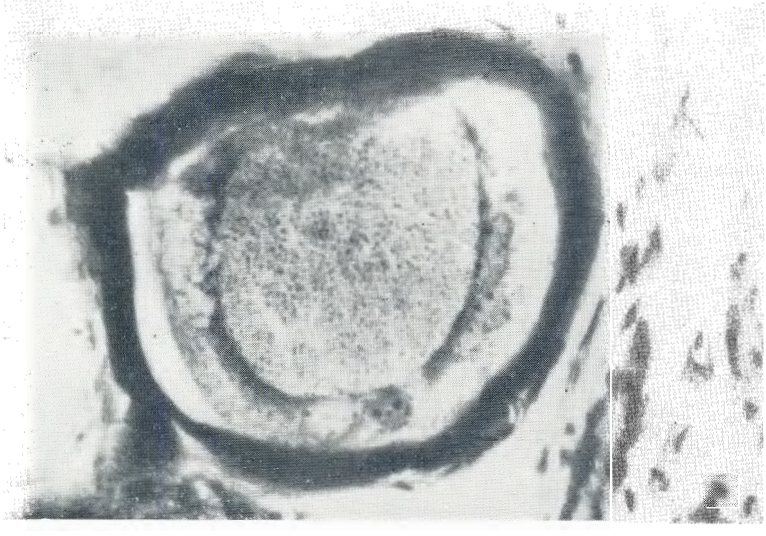

FIG. 16.—Immature cyst with distinct wall and inner membrane surrounded by distorted multinucleated histiocyte on 35th day × 480.

E.—Skin sections of cow No. 6547 prepared four months after natural infection. (Fig. 17 and 18)

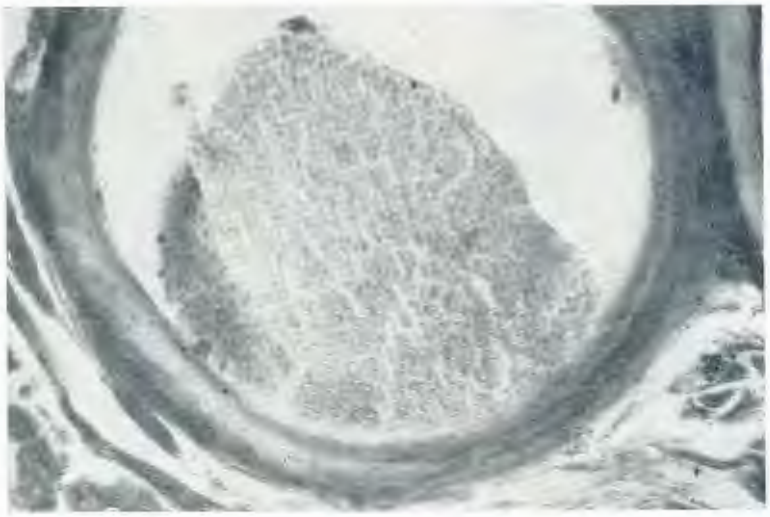

FIG. 17.—Mature cyst, showing well developed wall and numerous parasites × 480.

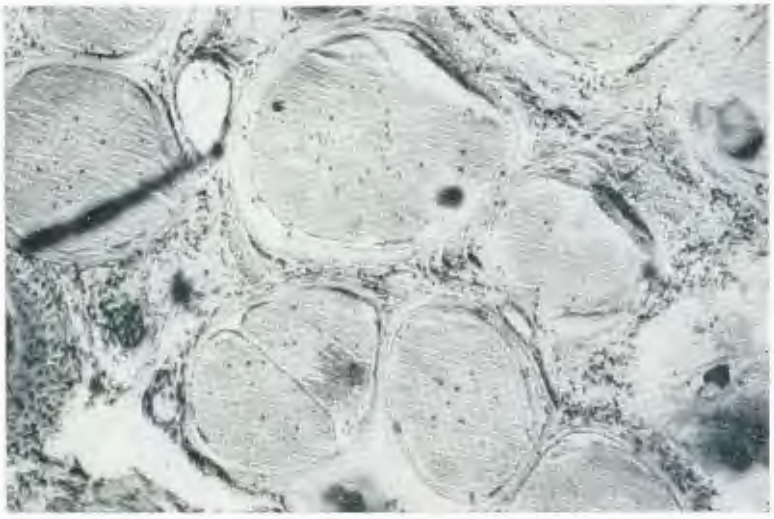

FIG. 18.—Group of mature cysts, one showing bilobular parasitic masses × 120.

F.—Skin sections of an artificially infected rabbit, No. 89, prepared 14 and 18 days after height of thermal reaction. (Fig. 19 and 20).

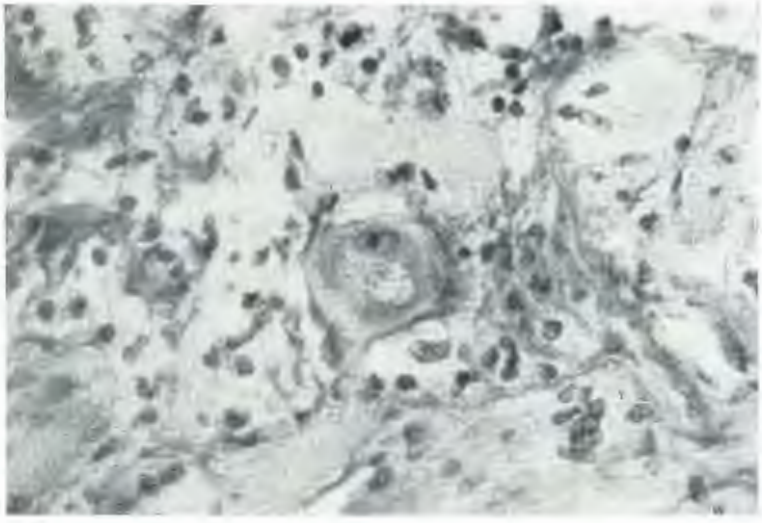

FIG. 19.—Uninucleated histiocyte harbouring a vacuole with parasites × 480.

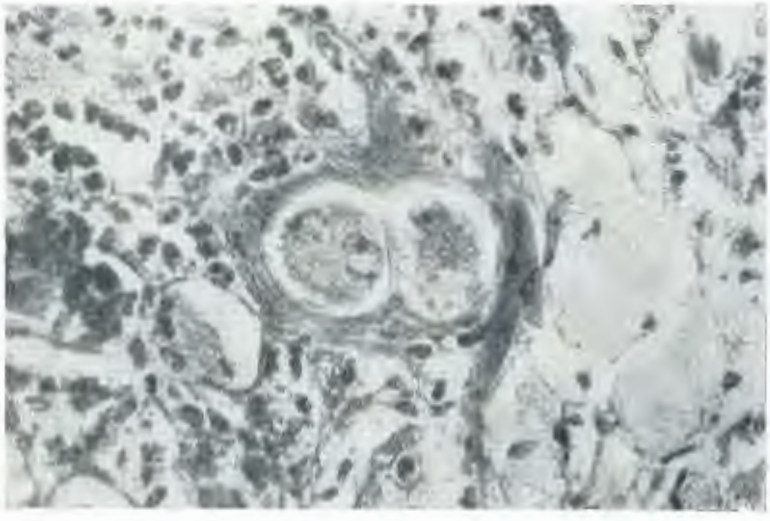

FIG. 20.—Two developing cysts harbouring a host cell nucleus, and vacuoles with parasites, and surrounded by connective tissue fibres × 480.

G.—Parasites, as seen in blood smears of different rabbits. (Fig. 21–24).

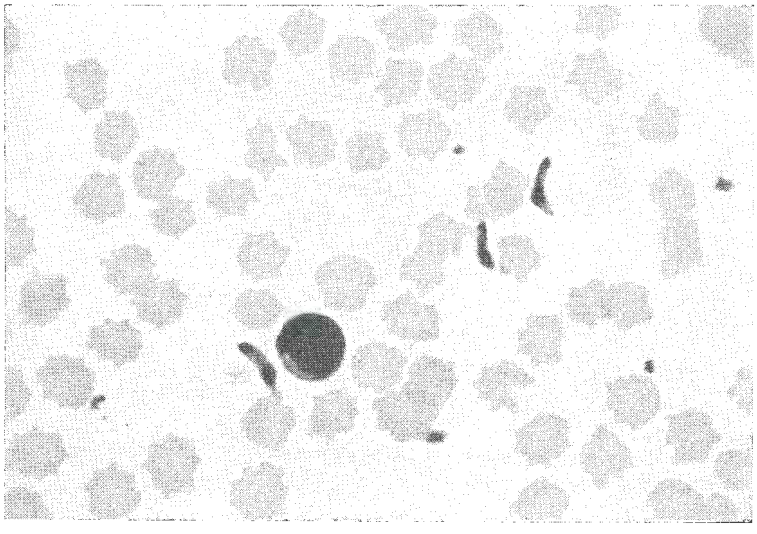

FIG. 21.—Contracted extracellular parasites × 1125.

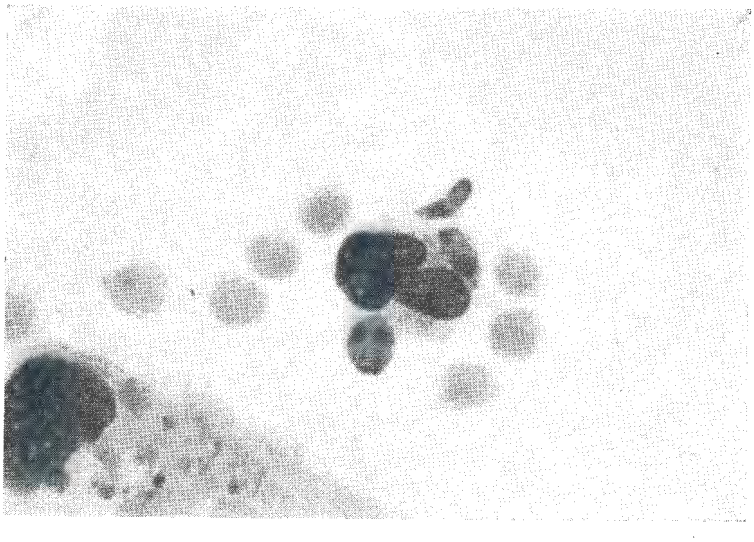

FIG. 22.—Monocyte with distorted cytoplasm and surrounded by parasites, one of which is binucleated and in the process of binary division. Note the darkly staining cap-like structure at polar end of the uninucleated free parasite × 1125.

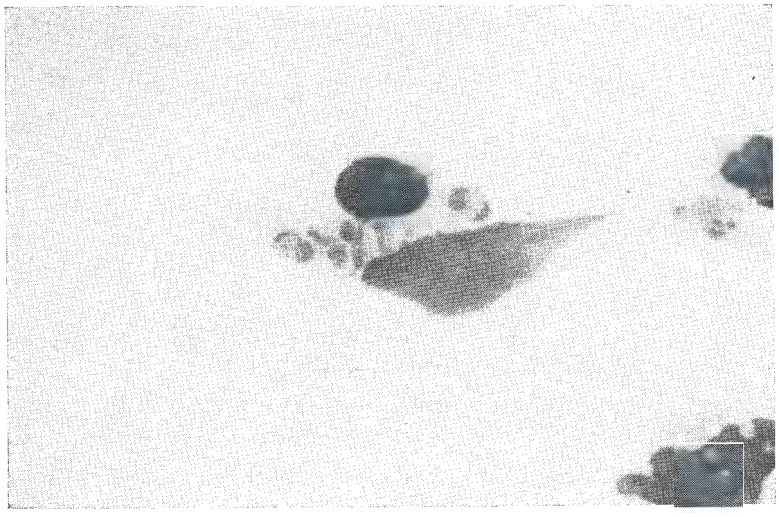

FIG. 23.—Monocyte with distorted cytoplasm and parasites in the process of reproduction. Note nuclear division in centrally situated parasite × 1125.

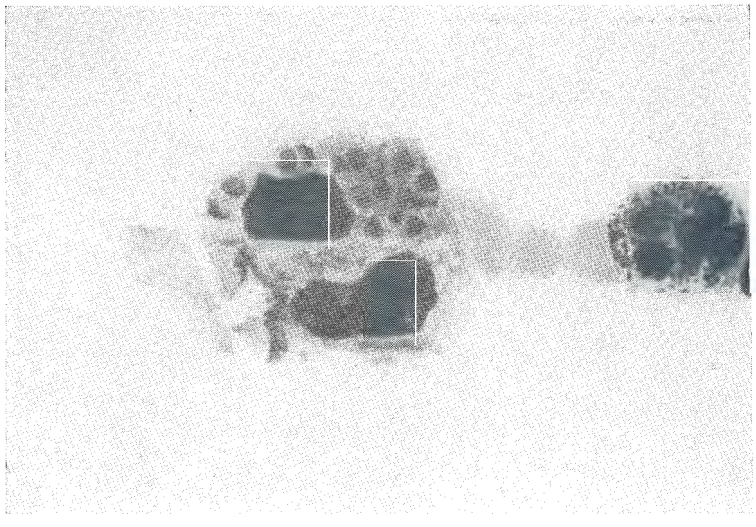

FIG. 24.—Monocyte harbouring parasites. Note schizont-like structure which probably represents an aberrant form. × 1125.

APPENDIX

TABLE A

Resistance of B. besnoiti *to physical and chemical agents*

Rabbit No.	Donor No.	Date of Infection	Dose of Blood in ml.	Stored Blood			Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Result
				Time	Max. Temp. °C	Anticoagulant		Date	Parasites	Oedema	Max. Temp. °F.			
344	338	6/10/54	4·0 ip..	0	39·0	Sodium citrate.....	6	18/10/54 19/10/54 20/10/54 21/10/54	+ + ++ ++	+	104·4	9	++	Died
346	338	7/10/54	5·0 ip..	0·5 hours.	20·0	Potassium oxalate.....	7	20/10/54 21/10/54	++ +++	+	105·4	8	++	Recovered
347	338	7/10/54	5·0 ip..	0	39·0	†Edington's solution....	15	2/11/54 3/11/54	+ ++	+	106·4	7	++	Recovered
348	338	7/10/54	5·0 ip..	0	39·0	†Edington's solution....	10	21/10/54 22/10/54	+++ +++	+++	105·4	5	+++	Died
1261	348	22/10/54	5·0 ip..	1·5 hours..	20·0	†Edington's solution....	—	—	—	—	103·0	—	—	No reaction
1262	348	22/10/54	5·0 ip..	1·5 hours..	20·0	†Edington's solution....	—	—	—	—	103·8	—	—	No reaction
1259	348	22/10/54	5·0 ip..	1·5 hours..	20·0	Sodium citrate.....	10	3/11/54	+	+	106·0	8	++	Recovered
1260	348	22/10/54	5·0 ip..	1·5 hours..	20·0	Sodium citrate.....	10	3/11/54 4/11/54 5/11/54	++ + +	+	106·8	7	++	Recovered
1257	344	22/10/54	5·0 ip..	24 hours..	20·0	Sodium citrate.....	5	1/11/54	+	+	105·8	8	++	Recovered
1258	344	22/10/54	5·0 ip..	24 hours..	20·0	Sodium citrate.....	10	3/11/54 4/11/54 5/11/54	+ + ++	+	105·6	7	++	Recovered
1267	344	2/11/54	4·0 ip..	12 days...	−76·0	Sodium citrate.....	—	—	—	—	103·0	—	—	No reaction
	1279	*1/12/54	5·0 ip..	0	39·0	Sodium citrate.....	9	14/12/54 15/12/54	+ ++	—	106·6	7	++	Recovered
1268	344	2/11/54	4·0 ip..	12 days...	−76·0	Sodium citrate.....	—	—	—	—	103·2	—	—	No reaction
1748	346	5/ 1/57	4·0 ip..	26 months.	−76·0	Sodium citrate.....	—	—	—	—	102·6	—	—	No reaction
1752	346	5/ 1/57	4·0 ip..	26 months.	−76·0	Sodium citrate.....	—	—	—	—	100·4	—	—	No reaction
1749	119	5/ 1/57	4·0 ip..	30 months.	−76·0	Sodium citrate.....	—	—	—	—	103·0	—	—	No reaction

* Immunity test. †Edington's solution = 500 ml. water
500 ml. glycerine
5·0 ml. carbolic acid
5·0 gm. potassium oxalate

‡ Antibiotics = 500 units of penicillin and 150 micrograms streptomycin per 1·0 ml. blood

TABLE A (*cont.*)

Rabbit No.	Donor No.	Date of Infection	Dose of Blood in ml.	Stored Blood			Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Result
				Time	Max. Temp. °C.	Anticoagulant		Date	Parasites	Oedema	Max. Temp. °F.			
1296	1289	20/12/54	5·0 ip..	0	39·0	Sodium citrate.....	9	3/ 1/55	++	—	106·0	9	++	Recovered
								4/ 1/55	+++					
1314	1296	6/ 1/55	5·0 ip..	48 hours..	20·0	Sodium citrate.....	12	24/ 1/55	+	+	106·2	7	++	Recovered
								25/ 1/55	++					
1316	1296	6/ 1/55	5·0 ip..	48 hours..	20·0	Sodium citrate and ‡antibiotics	—	—	—	—	103·0	—	—	No reaction
	1341	*4/ 3/55	5·0 ip..	0	39·0	Sodium citrate.....	5	10/ 3/55	+	+	104·8	9	+	Died
1317	1296	6/ 1/55	5·0 ip..	48 hours..	20·0	Sodium citrate and ‡antibiotics	—	—	—	—	106·6	—	—	No reaction
	1341	*4/ 3/55	5·0 ip..	0	39·0	Sodium citrate.....	7	16/ 3/55	+++	—	105·4	87	++	Died
								17/ 3/55	++					
1751	1296	5/ 1/57	4·0 ip..	24 months.	−76·0	Sodium citrate.....	—	—	—	—	103·0	—	—	No reaction
1307	1294	30/12/54	5·0 ip..	0	39·0	Sodium citrate.....	5	10/ 1/55	+++	+	104·8	7	++	Recovered
1319	1307	13/ 1/55	5·0 ip..	72 hours..	+ 4·0	Sodium citrate.....	7	24/ 1/55	++	+	105·8	4	++	Died
								25/ 1/55	+++					
1320	1307	14/ 1/55	5·0 ip..	72 hours..	+ 4·0	Sodium citrate and ‡antibiotics	8	24/ 1/55	+	+	106·2	6	++	Died
								25/ 1/55	+++					
1322	1307	14/ 1/55	5·0 ip..	96 hours..	+ 4·0	Sodium citrate.....	6	25/ 1/55	++	—	105·4	7	++	Died
								26/ 1/55	+++					
1323	1307	14/ 1/55	5·0 ip..	96 hours..	+ 4·0	Sodium citrate and ‡antibiotics	—	—	—	—	103·4	—	—	No reaction
	1341	*4/ 3/55	5·0 ip..	0	39·0	Sodium citrate.....	10	16/ 3/55	++	—	105·6	9	++	Recovered
1750	1307	5/ 1/57	4·0 ip..	24 months.	−76·0	Sodium citrate.....	—	—	—	—	103·0	—	—	No reaction
	1815	*19/ 3/57	5·0 ip..	0	39·0	Sodium citrate.....	8	31/ 3/59	+	+	105·6	7	++	Recovered
1482	1474	28/ 1/56	5·0 ip..	0·5 hours.	20·0	Heparin.....	4	2/ 2/56	+	+	105·6	6	++	Died
								3/ 2/56	+					
1486	1474	29/ 1/56	5·0 ip..	24 hours..	37·0	Sodium citrate.....	—	—	—	—	103·2	—	—	No reaction
1489	1474	31/ 1/56	5·0 ip..	96 hours..	20·0	Sodium citrate.....	—	—	—	—	104·0	—	—	No reaction
1535	1510	27/ 4/56	5·0 ip..	30 days...	−18·0	Sodium citrate.....	—	—	—	—	103·2	—	—	No reaction
	1552	*14/ 6/56	5·0 ip..	0	39·0	Sodium citrate.....	5	?	?	—	104·6	7	+	Recovered
1536	1510	27/ 4/56	5·0 ip..	30 days...	−18·0	Sodium citrate.....	—	—	—	—	104·0	—	—	No reaction
	1552	*14/ 6/56	5·0 ip..	0	39·0	Sodium citrate.....	6	25/ 6/56	++	+	105·8	8	++	Recovered
1803	1791	22/ 2/57	5·0 ip..	0	39·0	Sodium citrate and ‡antibiotics	14	14/ 3/57	+	—	106·4	10	++	Recovered
1804	1791	22/ 2/57	10·0 ip..	0	39·0	Sodium citrate and ‡antibiotics	8	6/ 3/57	+	—	105·4	6	++	Died

* Immunity test †Edington's solution = 500 ml. water
500 ml. glycerine
5·0 ml. carbolic acid
5·0 gm. potassium oxalate

‡ Antibiotics = 500 units of penicillin and 150 micrograms streptomycin per 1·0 ml. blood

TABLE B

Artificial transmission of B. besnoiti *to cattle*

Animal No.	Origin	Donor	Date of Infection	Dose of Blood ml. iv	Incubation Period in Days	Microscopical Observations				Clinical Symptoms	Remarks
						Trophozoites in Smears		Cysts in Skin Section			
						Date	Results	Date	Results		
Cow 6793..	Lydenburg. Received 31/1/53	Naturally infected ox which died from besnoitiosis	28/12/53	1,000	?	—	—	20/ 1/54	+	No fever or oedema of subcutaneous tissues	Very mild case
Ox 6840....	Rustenburg. Received 29/1/54. Natural case	—	—	—	—	1/2/54	Gland +	18/ 2/54	+	Fever 103·4° F. 28/1/54. Oedematous swelling of front limbs below the knees and dewlap	Relatively mild symptoms. Skin after 2 months showing scleroderma accompanied by depilation
Heifer 6839.	Rustenburg. Received 29/1/54	6840.....	28/ 1/54	1,000	10	8/2/54	Blood +	20/ 2/54	+	Febrile reaction for 3 days. Highest temperature 106·8° F. Oedematous swelling below the knees from 12/2/54 to 16/2/54	Mild reaction showing slight scleroderma and depilation
Bull 6851...	Pretoria district	—	—	—	—	4/2/54	Blood + Gland +	9/ 2/54	3+	Febrile reaction for 3 days. Highest temperature 106·6° F. Oedematous swelling of all limbs, dewlap and skin of thorax from 2/2/54 to 6/2/54	Acute reaction accompanied by anasarca and scleroderma. Died on 14/3/54 in a very cachectic condition
Ox 5895....	Available animal	6851.....	2/ 2/54	1,000	6	—	—	8/ 3/54	+	Febrile reaction for 3 days. Highest temperature 106° F. No swellings	Very mild case
Ox 5887....	Available animal	(R) R.T.....	15/ 2/54	13	7	—	—	10/ 3/54	+	No swellings noticed. Highest temperature 107° F	Mild reaction
Ox 5870....	Available animal	Ox 5887.....	25/ 2/54	250	9	6/3/54	Blood +	—	—	Oedema of front limbs. Highest temperature 105·8° F	—

+ = Parasites very rare. 2 + = Parasites fairly frequent. (R) = Rabbit.

TABLE C

Serial passage of the Fuls B. besnoiti *strain in rabbits*

Gen.	Donor	No. of Recipients	Date of Infection	Dose of Blood in ml.	No. that reacted	Serial No. of Rabbit selected as donor	History of Selected Donor								
							Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Died	Killed
								Date	Parasites	Oedema	Highest Temperature, °F				
1	Ox 6840	1	28/ 1/54	5·0 ip..	1	RB	3	10/ 2/54	+	++	—	3	++	+	—
2	RB	3	10/ 2/54	3·0 iv..	3	2	6	16/ 2/54 17/ 2/54	+ +	++	106·2	12	++	+	—
3	2	2	18/ 2/54	10·0 sc..	2	24	14	4/ 3/54 5/ 3/54 6/ 3/54	++ ++ ++	++	106·8	3	+++	+	—
4	24	2	4/ 3/54	2·5 sc..	2	39	13	18/ 3/54 19/ 3/54 20/ 3/54	+ ++ ++	+	107·0	3	++	+	—
5	39	2	19/ 3/54	2·5 ip..	2	54	8	29/ 3/54 31/ 3/54 1/ 4/54	+ + ++	+	106·2	4	++	+	—
6	54	6	31/ 3/54	2·0 ip..	5	58	6	7/ 4/54	++	+	106·6	4	++	+	—
7	58	2	7/ 4/54	2·0 ip..	2	67	6	15/ 4/54 20/ 4/54	+ ++	++	106·4	5	++	+	—
8	67	6	20/ 4/54	3·0 ip..	4	80	6	29/ 4/54 30/ 4/54 1/ 5/54	+ ++ +	+	105·6	5	++	+	—
9	80	4	29/ 4/54	2·5 ip..	4	83	7	10/ 5/54	+	—	105·6	—	+	—	+
10	83	6	10/ 5/54	2·5 ip..	5	96 98	8 9	19/ 5/54 19/ 5/54 20/ 5/54	+ ++ +	— —	105·8 105·2	— —	+ +	— —	+ +
11	96 & 98	3	19/ 5/54	2·5 ip..	2	106	13	5/ 6/54 8/ 6/54 10/ 6/54	+ + +	+	106·0	38	+	+	—
12	106	2	5/ 6/54	4·0 ip..	1	109	7	15/ 6/54 16/ 6/54 17/ 6/54 18/ 6/54	+ ++ + +	++	105·6	12	++	+	—

TABLE C (*continued*)

Gen.	Donor	No. of Recipients	Date of Infection	Dose of Blood in ml.	No. that reacted	Serial No. of Rabbit selected as donor	History of Selected Donor								
							Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Died	Killed
								Date	Parasites	Oedema	Highest Temperature, °F				
13	109	2	12/ 6/54 15/ 6/54 15/ 6/54	4·0 ip.. 2·0 ip.. 4·0 ip..	2	112 114	9 7	28/ 6/54 28/ 6/54	+ ++	−	105·8 105·6	7 6	++ ++	+	−
14	112 & 114	4	28/ 6/54	5·0 ip..	2	115	7	3/ 7/54 5/ 7/54	+ +	++	106·4	8	++	+	−
15	115	2	5/ 7/54	5·0 ip..	2	119	5	14/ 7/54	++	+	106·2	5	++	+	−
16	119	1	14/ 7/54	6·0 ip..	1	304	5	26/ 7/54	++	+	106·0	3	++	+	−
17	304	2	26/ 7/54	5·0 ip..	2	305 306	6 8	− −	− −	++ +	106·6 105·6	5 6	++ +	+ +	− −
18	305 & 306	2	17/ 8/54	1 0·0 ip.	2	316	12	31/ 8/54	+	−	104·8	5	+	+	−
19	316	3	31/ 8/54	5·0 ip..	2	320	13	13/ 9/54	+	+	105·2	4	++	+	−
20	320	3	11/ 9/54	4·0 ip..	3	326	9	23/ 9/54	++	+	106·0	9	++	+	−
21	326	3	23/ 9/54	4·0 ip..	2	333 334	8 7	5/10/54 6/10/54	++ +	++ +	105·8 105·0	4 15	++ +	+ +	− −
22	333 & 334	3	5/10/54	5·0 ip..	3	339	8	16/10/54 18/10/54 19/10/54	++ ++ +++	++	105·2	6	+++	+	−
23	339	4	16/10/54	4·0 ip..	4	1255	6	26/10/54 27/10/54 28/10/54 29/10/54	++ ++ +++ +++	+++	106·0	7	+++	+	−
24	1255	4	27/10/54	5·0 ip..	4	1264	6	6/11/54 8/11/54	+ ++	+	106·6	6	++	+	−
25	1264	5	8/11/54	5·0 ip..	3	1273	8	19/11/54 20/11/54	+ +++	+	105·6	6	++	+	−
26	1273	5	19/11/54	5·0 ip..	3	1279	7	27/11/54 29/11/54 1/12/54	+ ++ +++	+	106·6	5	++	+	−

TABLE C (*continued*)

Gen.	Donor	No. of Recipients	Date of Infection	Dose of Blood in ml.	No. that reacted	Serial No. of Rabbit selected as donor	History of Selected Donor								
							Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Died	Killed
								Date	Parasites	Oedema	Highest Temperature, °F				
27	1279	4	29/11/54	5·0 ip..	4	1286	7	7/12/54 8/12/54 9/12/54 10/12/54	+ ++ ++ +++	+	105·4	4	+++	+	−
28	1286	4	10/12/54	5·0 ip..	4	1291	4	17/12/54 18/12/54	++ ++	−	104·6	6	++	+	−
29	1291	4	18/12/54	5·0 ip..	4	1294	6	28/12/54 30/12/54	+++ +	−	105·8	8	++	+	−
30	1294	4	30/12/54	5·0 ip..	4	1305	8	7/ 1/55 8/ 1/55	+ ++	+	105·6	2	++	+	−
31	1305	4	8/ 1/55	5·0 ip..	4	1311	6	18/ 1/55 20/ 1/55 21/ 1/55	+ ++ +	+	105·6	9	++	+	−
32	1311	3	20/ 1/55	5·0 ip..	3	1327	10	31/ 1/55 1/ 2/55	++ +++	−	106·2	3	++	+	−
33	1327	4	1/ 2/55	5·0 ip..	4	1338	8	11/ 2/55 12/ 2/55	++ +	−	104·2	8	++	+	−
34	1338	3	11/ 2/55	5·0 ip..	2	1344	8	25/ 2/55	++	−	106·2	?	++	−	+
35	1344	4	25/ 2/55	5·0 ip..	4	1349	6	5/ 3/55	+	+	106·0	?	++	−	+
36	1349	3	7/ 3/55	5·0 ip..	3	1353	6	17/ 3/55 18/ 3/55	++ +	+	106·4	6	++	+	−
37	1353	3	17/ 3/55	5·0 ip..	3	1356	5	26/ 3/55 28/ 3/55 29/ 3/55	++ + +	+	106·4	8	++	+	−
38	1356	3	26/ 3/55	5·0 ip..	3	1360	8	4/ 4/55 7/ 4/55	+ +++	−	106·6	3	+++	+	−
39	1360	3	7/ 4/55	4·0 ip..	3	1361	4	15/ 4/55 16/ 4/55	+ ++	+	105·6	6	++	+	−

TABLE C (*continued*)

Gen.	Donor	No. of Recipients	Date of Infection	Dose of Blood in ml.	No. that reacted	Serial No. of Rabbit selected as donor	History of Selected Donor								
							Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Died	Killed
								Date	Parasites	Oedema	Highest Temperature, °F				
40	1361	3	16/ 4/55	4 0 ip..	3	1365	7	25/ 4/55 26/ 4/55 27/ 4/55	+ ++ ++	−	105·0	3	++	+	−
41	1365	4	26/ 4/55	4·0 ip..	4	1369	6	5/ 5/55 6/ 5/55	+ +	+	105·6	8	++	+	−
42	1369	4	6/ 5/55	5·0 ip..	4	1376	9	20/ 5/55	+	−	105·8	13	+	+	−
43	1376	4	20/ 5/55	5·0 ip..	3	1379	8	30/ 5/55	+	+	105·4	4	++	+	−
44	1379	4	30/ 5/55	5·0 ip..	3	1383	5	9/ 6/55	+	+	107·0	?	+	−	+
45	1383	4	10/ 6/55	5·0 ip..	4	1388	13	28/ 6/55	+	+	105·8	6	++	+	−
46	1388	4	28/ 6/55	5·0 ip..	2	1389	5	5/ 7/55	+	+	105·4	10	++	+	−
47	1389	4	6/ 7/55	5·0 ip..	2	1394	7	18/ 7/55	+	+	105·8	5	++	+	−
48	1394	4	18/ 7/55	5·0 ip..	2	1397	9	30/ 7/55	+	+	105·8	5	++	+	−
49	1397	4	1/ 8/55	5·0 ip.. 5·0 ip..	2	1401 1403	9 10	− 9/ 8/55	− +	− −	105·0 105·0	8 5	+ ++	+ +	− −
50	1401 & 1403	4	12/ 8/55	5·0 ip..	3	1406	8	22/ 8/55 23/ 8/55	+ ++	+	107·0	5	++	+	−
51	1406	4	23/ 8/55	5·0 ip..	4	1410	8	6/ 9/55	+	−	106·6	10	++	+	−
52	1410	4	6/ 9/55	5·0 ip..	1	1414	7	16/ 9/55	+	−	107·0	6	++	+	−
53	1414	4	16/ 9/55	5·0 ip..	3	1417	7	26/ 9/55	+	+	105·2	4	++	+	−
54	1417	4	27/ 9/55	5·0 ip..	3	1424	10	8/10/55	+	−	105·6	6	++	+	−
55	1424	4	8/10/55	5·0 ip..	4	1427	8	17/10/55 18/10/55	+ ++	+	104·2	3	++	+	−
56	1427	4	18/10/55	5·0 ip..	4	1431	10	29/10/55	+	−	106·0	4	++	+	−
57	1431	4	29/10/55	5·0 ip..	3	1435	10	10/11/55	++	−	105·8	4	++	+	−

TABLE C (*continued*)

Gen.	Donor	No. of Recipients	Date of Infection	Dose of Blood in ml.	No. that reacted	Serial No. of Rabbit selected as donor	History of Selected Donor								
							Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Died	Killed
								Date	Parasites	Oedema	Highest Temperature, °F				
58	1435	4	10/11/55	5·0 ip..	3	1439	7	19/11/55	++	–	106·4	?	++	–	+
59	1439	4	19/11/55	5·0 ip..	4	1441	6	28/11/55	++	–	106·8	6	+++	+	–
60	1441	4	29/11/55	5·0 ip..	4	1446	6	5/12/55 6/12/55 7/12/55 8/12/55	+ + ++ +++	+	106·6	6	+++	+	–
61	1446	4	6/12/55	5·0 ip.	4	1451	7	14/12/55 15/12/55	+ ++	–	107·8	?	++	–	+
62	1451	4	15/12/55	5·0 ip..	4	1453	7	23/12/55	++	+	105·6	3	++	+	–
63	1453	4	24/12/55	3·0 ip..	4	1459	6	3/ 1/56	++++	+	106·6	?	++	–	+
64	1459	4	3/ 1/56	3·0 ip..	4	1468	9	16/ 1/56	+	+	105·0	?	++	–	+
65	1468	4	16/ 1/56	5·0 ip..	2	1475	7	24/ 1/56	+	–	106·0	?	++	–	+
66	1475	4	26/ 1/56	5·0 ip..	2	1478	7	4/ 2/56	++	–	105·2	?	++	–	+
67	1478	4	4/ 2/56	5·0 ip..	4	1493	7	15/ 2/56	++	+	105·0	?	++	–	+
68	1493	4	15/ 2/56	5·0 ip..	3	1503	10	1/ 3/56	+++	–	105·6	?	++	–	+
69	1503	3	1/ 3/56	5·0 ip..	2	1509	12	16/ 3/56	+	+	104·4	?	++	–	+
70..	1509	2	16/ 3/56	5·0 ip..	2	1514	6	23/ 3/56 24/ 3/56	+ ++	–	105·8	4	++	+	–
71	1514	4	23/ 3/56	5·0 ip..	4	1517	7	4/ 4/56	+	+	105·6	?	++	–	+
72	1517	4	5/ 4/56	5·0 ip..	4	1523	7	16/ 4/56 17/ 4/56	+ +++	++	106·2	?	++	–	+
73	1523	4	16/ 4/56	5·0 ip..	4	1527	8	28/ 4/56 2/ 5/56	+ +	–	104·8	?	++	–	+
74	1527	3	28/ 4/56	5·0 ip..	2	1537	15	18/ 5/56	+	+	105·0	?	++	–	+
75	1537	2	19/ 5/56	5·0 ip.	1	1556	8	30/ 5/56	+	–	105·8	?	++	–	+

TABLE C (*continued*)

Gen.	Donor	No. of Recipients	Date of Infection	Dose of Blood in ml.	No. that reacted	Serial No. of Rabbit selected as donor	History of Selected Donor								
							Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Died	Killed
								Date	Parasites	Oedema	Highest Temperature, °F				
76	1556	4	30/ 5/56	5·0 ip..	4	1563	9	15/ 6/56	+	–	105·6	?	++	–	+
77	1563	4	15/ 6/56	5·0 ip..	4	1581	6	29/ 6/56	++	+	106·2	?	++	–	+
78	1581	3	29/ 6/56	5·0 ip..	3	1590	8	12/ 7/56	+	++	107·6	4	+++	+	–
79	1590	4	12/ 7/56	5·0 ip..	4	1597	5	24/ 7/56	++	+	105·2	3	++	+	–
80	1597	4	24/ 7/56	5·0 ip..	4	1607 1608	7 8	7/ 8/56 8/ 8/56	+ +	– –	104·0 104·6	? ?	+ +	– –	+ +
81	1607 & 1608	4	8/ 8/56	5·0 ip..	2	1614	7	20/ 8/56	+++	–	105·0	?	++	–	+
82	1614	1	20/ 8/56	5·0 ip..	1	1633	7	31/ 8/56	++	+	106·0	4	++	+	–
83	1633	2	31/ 8/56	5·0 ip..	2	1641	5	14/ 9/56	++	–	105·0	?	++	–	+
84	1641	3	14/ 9/56	5·0 ip..	3	1650	6	26/ 9/56	++	–	105·8	?	++	–	+
85	1650	2	26/ 9/56	5·0 ip..	2	1668	5	8/10/56	+++	++	105·4	3	+++	+	–
86	1668	4	8/10/56	5·0 ip..	4	1681	5	16/10/56	+++	–	105·4	?	++	+	–
87	1681	4	16/10/56	5·0 ip..	3	1688	6	27/10/56	+	+	106·0	?	+	–	+
88	1688	4	27/10/56	5·0 ip..	4	1695	8	9/11/56	++	–	104·4	?	+	–	+
89	1695	4	9/11/56	5·0 ip..	2	1704	8	21/11/56	++	+	106·2	?	++	–	+
90	1704	4	21/11/56	5·0 ip..	2	1716	8	29/11/56	++	+	106·0	3	++	+	–
91	1716	4	29/11/56	5·0 ip..	4	1722	7	10/12/56	+	–	104·4	?	+	–	+
92	1722	4	10/12/56	5·0 ip..	3	1730	7	20/12/56	+	–	105·8	?	+	–	+
93	1730	4	20/12/56	5·0 ip..	3	1737	6	2/ 1/57	+++	–	104·8	?	++	–	+
94	1737	3	2/ 1/57	5·0 ip..	1	1745	10	21/ 1/57	++	–	104·4	?	+	–	+

TABLE C (*continued*)

Gen.	Donor	No. of Recipients	Date of Infection	Dose of Blood in ml.	No. that reacted	Serial No. of Rabbit selected as donor	History of Selected Donor								
							Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Died	Killed
								Date	Parasites	Oedema	Highest Temperature, °F				
95	1745	2	21/ 1/57	5·0 ip..	2	1763	8	4/ 2/57 5/ 2/57	++ ++	+	105·2	3	++	+	−
96	1763	4	4/ 2/57	5·0 ip..	1	1777	4	14/ 2/57	++++	−	105·0	?	++	−	+
97	1777	4	14/ 2/57	5·0 ip..	1	1791	3	22/ 2/57	+	−	105·8	?	++	−	+
98	1791	2	22/ 2/57	5·0 ip..	2	1801	4	5/ 3/57	++	+	105·6	?	++	−	+
99	1801	4	5/ 3/57	5·0 ip..	3	1813	6	15/ 3/57	+	−	106·8	?	++	−	+
100	1813	4	15/ 3/57	5·0 ip..	2	1821	7	26/ 3/57	++	−	105·4	?	++	−	+
101	1821	4	26/ 3/57	5·0 ip..	2	1830	9	8/ 4/57	++	−	103·6	?	++	−	+
102	1830	4	8/ 4/57	5·0 ip..	3	1836	8	23/ 4/57	++	++	108·0	10	+++	+	−
103	1836	4	23/ 4/57	5·0 ip..	2	1839	5	3/ 5/57	+	+	104·6	?	++	−	+
104	1839	4	3/ 5/57	5·0 ip..	4	1847	6	13/ 5/57	++	−	105·5	?	++	−	+
105	1847	4	13/ 5/57	5·0 ip..	3	1852	7	24/ 5/57	+	−	105·4	?	++	−	+
106	1852	4	24/ 5/57	5·0 ip..	3	1857	7	4/ 6/57	++	−	106·0	?	++	−	+
107	1857	4	4/ 6/57	5·0 ip..	4	1861	7	13/ 6/57	+	−	105·4	?	++	−	+
108	1861	4	13/ 6/57	5·0 ip..	3	1865	5	25/ 6/57	++	−	105·8	?	++	−	+
109	1865	4	25/ 6/57	5·0 ip..	3	1870	6	10/ 7/57	+	−	105·0	?	++	−	+
110	1870	3	10/ 7/57	5·0 ip..	1	1879	4	17/ 7/57	+++	−	106·4	9	++	+	−
111	1879	4	17/ 7/57	5·0 ip..	4	1884	7	29/ 7/57	+	−	105·4	?	++	−	+
112	1884	2	29/ 7/57	5·0 ip..	2	1891	6	8/ 8/57	+	+	105·8	?	++	−	+
113	1891	2	8/ 8/57	5·0 ip..	2	1902	5	21/ 8/57	++	−	105·2	?	++	−	+
114	1902	4	21/ 8/57	5·0 ip..	4	1908	6	31/ 8/57	+	+	105·8	?	++	−	+

TABLE C (*continued*)

Gen.	Donor	No. of Recipients	Date of Infection	Dose of Blood in ml.	No. that reacted	Serial No. of Rabbit selected as donor	History of Selected Donor							
							Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Died	Killed
								Date	Parasites	Oedema	Highest Temperature, °F.				
115	1908	4	31/ 8/57	5·0 ip..	3	1917	7	11/ 9/57	+	–	106·4	?	++	–	+
116	1917	4	11/ 9/57	5·0 ip..	4	1921	5	20/ 9/57	+	–	106·0	?	++	–	+
117	1921	4	20/ 9/57	5·0 ip..	4	1924	7	1/10/57	+	–	106·2	?	++	–	+
118	1924	4	1/10/57	5·0 ip..	1	1933	6	12/10/57	+	–	105·8	?	++	–	+
119	1933	4	12/10/57	5·0 ip..	3	1939	5	23/10/57	+	–	106·0	?	++	–	+
120	1939	2	23/10/57	5·0 ip..	1	1944	6	2/11/57	++	+	106·2	?	++	–	+
121	1944	2	2/11/57	5·0 ip..	1	1948	6	13/11/57	++	–	105·4	10	++	+	–
122	1948	4	13/11/57	5·0 ip..	2	1953	7	26/11/57	+	–	105·4	?	++	–	+
123	1953	2	27/11/57	5·0 ip..	1	1961	3	4/12/57	+	–	104·8	?	+	–	+
124	1961	4	4/12/57	5·0 ip..	2	1965	9	17/12/57	+	–	105·4	?	++	–	+
125	1965	4	17/12/57	5·0 ip..	2	1970	7	30/12/57	+++	–	105·2	?	++	–	+

TABLE D

Observations on artificially infected goats and sheep

Gen.	Animal No.	Donor	Date of Infection	Blood iv.	Incubation Period in Days	Examination of Blood Smears	Clinical Symptoms		Remarks	Biological Test on Rabbits	
							Oedema	Highest Temperature, °F.		Rabbit No.	Severity of Reaction
1	G. 79328..	R. 327.... (Gen. 20)	28/9/54	35·0 ml.	8	—	++	107·0	Alopecia in anterior half of body. Observed for six months and recovered. Cysts seen in skin sections	R. 1251. R. 1252.	++ ++
1	G. 88537..	R. 1468... (Gen. 64)	16/1/56	30·0 ml.	5	—	—	106·2	Observed for three-and-a-half months and killed. Cysts seen in skin sections	R. 1476. R. 1484.	++ ++
2	G. 88549..	G. 88537..	25/1/56	50·0 ml.	4	—	—	106·6	Died of acute peritonitis a month later. No cysts seen in skin sections	R. 1491.	++
3	G. 88536..	G. 88549..	3/2/56	50·0 ml.	7	—	—	106·8	Died from haemorrhagic enteritis two-and-a-half months later. No cysts seen in skin sections	R. 1499. R. 1500.	++ ++
1	Sh. 86569.	R. 327.... (Gen. 20)	28/9/54	35·0 ml.	7	—	—	107·0	Was challenged 28/11/56 (Donor R. 1441, Gen. 59). No reaction noted. Killed 2/5/56. No cysts seen in skin sections	R. 349.. R. 350..	++ ++
1	Sh. 89632.	R. 1468... (Gen. 64)	16/1/56	30·0 ml..	5	—	—	107·2	Observed for three-and-a-half months. Aborted eight weeks after highest temperature. Killed 2/5/56. No cysts in skin sections		
2	Sh. 615...	Sh. 89632.	25/1/56	30·0 ml..	6	—	—	106·8	Observed for three-and-a-half months. Killed 2/5/56. No cysts in skin sections	R. 1492.	++
3	Sh. 145...	Sh. 615...	3/2/56	50·0 ml..	7	—	—	106·8	Observed for three-and-a-half months. Killed 2/5/56. No cysts seen in skin sections	R. 1497. R. 1498.	++ ++

G. = Goat; Sh. = Sheep; iv. = intravenously; + = mild; ++ = severe

TABLE E

Duration of blood parasitaemia in artificially B. besnoiti *infected rabbits*

Rabbit No.	Donor No.	Date of Infection	No. of Days after Infection	Dose of Blood i.p. in ml.	Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Result
						Date	Parasites	Oedema	Max. Temp., °F			
1318	1307	11/ 1/55	—	5·0	5	21/ 1/55 22/ 1/55	+ +	+	106·6	8	++	Recovered
1321	1318	13/ 1/55	2	5·0	18	7/ 2/55 8/ 2/55 9/ 2/55	++ +++ +++	—	106·4	10	+++	Died
1324	1318	15/ 1/55	4	5·0	12	29/ 1/55 31/ 1/55	+ ++	+	106·0	5	++	Died
1325	1318	17/ 1/55	6	5·0	12	1/ 2/55	+	+	105·8	4	++	Died
1326	1318	19/ 1/55	8	5·0	8	31/ 1/55 1/ 2/55	++ +++	+	107·0	6	++	Died
1330	1318	21/ 1/55	10	5·0	9	3/ 2/55 4/ 2/55 5/ 2/55	+ + +	+	106·0	8	++	Died
1331	1318	23/ 1/55	12	5·0	7	2/ 2/55 3/ 2/55 4/ 2/55 5/ 2/55	+ ++ + ++	+	106·2	9	++	Died
1332	1318	25/ 1/55	14	5·0	9	8/ 2/55 9/ 2/55 10/ 2/55	++ +++ +++	+++	107·4	8	+++	Died
1333	1318	27/ 1/55	16	5·0	11	12/ 2/55 14/ 2/55	+ +	+	106·0	9	++	Died
1334	1318	29/ 1/55	18	5·0	14	17/ 2/55 18/ 2/55	++ ++	++	106·6	7	+++	Died

*Immunity test

Parasites + very rare, ++ rare, +++ infrequent

Oedema + = mild, ++ = fairly severe, +++ = very severe

Severity of reaction ++ = fairly severe, +++ = severe

TABLE E (*continued*)

Rabbit No.	Donor No.	Date of Infection	No. of Days after Infection	Dose of Blood i.p. in ml.	Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Result
						Date	Parasites	Oedema	Max. Temp. °F			
1335	1318	31/ 1/55	20	5·0	21	28/ 2/55 1/ 3/55	++ +	+	105·6	8	++	Recovered
1340	1318	4/ 2/55	22	5·0	18	23/ 2/55	+	–	105·2	7	++	Recovered
1341	1318	4/ 2/55	24	5·0	23	2/ 3/55 3/ 3/55 4/ 3/55	+ ++ +++	+	105·8	10	++	Recovered
1436	1431	29/10/55	—	5·0	12	15/11/55 16/11/55	++ ++	–	106·0	10	++	Recovered
1457	1436	15/12/55	47	5·0	–	–	–	–	103·8	–	–	No reaction
	1493	*15/ 2/56		5·0	8	22/ 2/56	+	–	106·0	8	++	Recovered
1458	1436	15/12/55	47	5·0	–	–	–	–	103·0	–	–	Died during incubation period

* Immunity test

Parasites + very rare, ++ rare, +++ infrequent

Oedema + = mild, ++ = fairly severe, +++ = very severe

Severity of reaction ++ = fairly severe, +++ = severe

TABLE F

Transmission of B. besnoiti *by means of materials, other than blood, from affected rabbits*

Rabbit No.	Donor No.	Date of Infection	Material used	Dose in ml.	Route of Administration	Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Severity of Reaction	Result
							Date	Parasites	Oedema	Max. Temp., °F			
13	RT.....	15/ 2/54	Oedematous fluid.....	5·0	sc.....	6	25/ 2/54 26/ 2/54	+ +	+	106·2	5	++	Died
1308	1296	4/ 1/55	Spleen suspension....	5·0	ip.....	7	15/ 1/55 17/ 1/55 18/ 1/55 20/ 1/55	+ ++ ++ +	+	106·6	7	+++	Recovered
1309	1296	4/ 1/55	Spleen suspension....	5·0	ip.....	7	15/ 1/55 16/ 1/55 17/ 1/55	+++ +++ +	+++	107·0	5	+++	Recovered
1654	1641, 1643	14/ 9/56	Urine and faeces.....	20·0	per os...	—	—	—	—	103·0	—	—	No reaction
1655	1641, 1643	14/ 9/56	Urine and faeces.....	20·0	per os...	—	—	—	—	103·6	—	—	No reaction
1656	1641, 1643	14/ 9/56	Urine and faeces.....	20·0	per os...	—	—	—	—	103·2	—	—	No reaction
1657	1641, 1643	14/ 9/56	Urine and faeces.....	20·0	per os...	—	—	—	—	103·2	—	—	No reaction
1659	1641, 1643	14/ 9/56	Liver and spleen suspension	5·0	ip.....	10	30/ 9/56 1/10/56	++ ++	+	105·6	8	++	Recovered
1660	1641, 1643	14/ 9/56	Liver and spleen suspension	10·0	per os...	—	—	—	—	102·8	—	—	No reaction
	1681	*16/10/56	Blood.....	5·0	ip.....	6	23/10/56	+	—	106·0	8	++	Recovered
1661	1641, 1643	14/ 9/56	Liver and spleen suspension	10·0	per os...	—	—	—	—	103·2	—	—	No reaction
	1681	*16/10/56	Blood.....	5·0	ip.....	7	24/10/56	+	+	106·4	7	++	Recovered
1675	1668	8/10/56	Faeces and urine.....	30·0	per os...	—	—	—	—	104·0	—	—	No reaction
	1695, 1696	*9/11/56	Blood.....	5·0	ip.....	8	21/11/56	+++	+	106·0	8	++	Recovered
1676	1668	8/10/56	Faeces and urine.....	30·0	per os...	—	—	—	—	103·4	—	—	No reaction
	1696, 1696	*9/11/56	Blood.....	5·0	ip.....	6	21/11/56 22/11/56	+++ ++	—	106·2	9	++	Recovered

* Immunity test

Parasites + = very rare, ++ = rare, +++ = infrequent

Oedema + = mild, ++ = fairly severe

Severity of reaction ++ = fairly severe, +++ = severe

TABLE G

Spermatogenesis in artificially infected ruminants before and after recovery

Animal	Donor	Date of Infection	Dose of Blood in ml.	Incubation Period in Days	Examination of Semen					Remarks
					Date	Ejaculate in ml.	Motility	Percentage Live Sperms	Percentage Abnormal Sperms	
Bull No. 5873..	Rabbit No. 1522	24/ 4/56	35·0 iv..	6	24/ 4/56	8·0	Good...	68	5	Developed shock after blood injection
					27/ 4/56	8·0	Good...	68	5	Highest temperature recorded 106·0°F
					1/ 5/56	5·0	0	0	0	
					8/ 5/56	6·0	0	0	All dead	
					15/ 5/56	5·5	0	0	All dead	
					22/ 5/56	4·0	0	0	All dead	
					28/ 5/56	4·0	0	0	No sperms	
					5/ 6/56	6·0	0	0	No sperms	*Trichomonas foetus* present
					12/ 6/56	7·0	0	0	No sperms	,, ,, ,,
					19/ 6/56	8·0	0	0	Few dead	,, ,, ,,
					26/ 6/56	8·0	0	0	Few dead	,, ,, ,,
					10/ 8/56	10·0	0	5	5	,, ,, ,,
					14/ 9/56	12·0	0	5	5	,, ,, ,,
					13/10/56	10·0	Poor....	12	12	,, ,, ,,
					30/10/56	7·0	Fair.....	50	0	,, ,, ,,
					6/12/56	8·0	Fair.....	55	0	,, ,, ,,
					21/12/56	—	—	—	—	,, ,, ,,
	Rabbit No. 1846	15/ 5/57*	35·0 iv..	—	—	—	—	—	—	—
					22/ 5/57	5·0	Good...	60	5	*Trichomonas foetus* absent
					29/ 5/57	6·0	Fair.....	63	5	,, ,, ,,
					7/ 6/57	6·0	Fair.....	65	0	,, ,, ,,
					13/ 6/57	7·0	Fair.....	61	0	,, ,, ,,
					20/ 6/57	6·0	Fair.....	66	5	,, ,, ,,
					4/ 7/57	7·0	Fair.....	63	0	,, ,, ,,
					8/ 8/57	7·0	Fair.....	75	0	,, ,, ,,
	Rabbit No. 2350	10/ 4/59*	30·0 sc..	—	10/ 4/59	—	—	—	—	No clinical symptoms seen
Bull No. 5981...	Rabbits No. 1512 and 1513	26/ 3/56	30·0 iv..	7	12/ 3/56	5·0	Good...	65	0	Highest temperature recorded 106·2°F
					15/ 3/56	5·0	Good...	65	0	Animal listless during period of reaction
					10/ 4/56	5·0	0	0	0	
					17/ 4/56	5·0	0	0	60	Left testicle swollen and hard
					23/ 4/56	3·5	0	0	60	Swelling increased; animal dejected and shows stiff gait
					1/ 5/56	4·0	0	0	No sperms	
					8/ 5/56	3·0	0	0	No sperms	
					15/ 5/56	4·5	0	0	No sperms	
					22/ 5/56	3·0	0	0	No sperms	
					12/ 6/56	6·0	0	0	Few dead	
					19/ 6/56	8·0	0	0	Few dead	
					26/ 6/56	6·0	0	0	Few dead	Abscess opened; diagnosis *Corynebacterium* infection
					10/ 8/56	6·0	0	27	50	
					14/ 9/56	12·0	Good...	70	50	
					13/10/56	7·0	Good...	65	0	Left testicle fibrotic
					30/10/56	10·0	Fair.....	63	0	
					6/12/56	6·0	Fair.....	75	0	

* = Immunity Test

TABLE G (*continued*)

Animal	Donor	Date of Infection	Dose of Blood in ml.	Incubation Period in Days	Examination of Semen					Remarks
					Date	Ejaculate in ml.	Motility	Percentage Live Sperms	Percentage Abnormal Sperms	
Billy Goat No. 3171	Rabbit No. 1922	21/ 9/57	30·0 iv..	6	21/ 9/57	1·0	Very good	76	0	Highest temperature recorded 106·8°F. Animal was listless, and showed inappetence for three days after height of thermal reaction
					7/10/57	1·5	Poor....	36	50	
					14/10/57	1·0	Poor....	38	50	
					23/10/57	1·0	0	0	No sperms	
					28/10/57	1·0	0	0	No sperms	
					11/11/57	0·5	0	0	No sperms	
					19/11/57	2·0	Poor....	30	59	
					2/12/57	1·0	Fair.....	49	49	
					6/ 1/58	2·0	Good...	70	0	
	Rabbit No. 1977	23/ 1/58*	30·0 iv.	—	7/ 2/58	2·5	Good...	78	0	
					18/ 2/58	1·0	Good...	75	0	
	Rabbit No. 2307	19/ 3/59*	30·0 iv..	—	—	—	—	—	—	No clinical symptoms
Ram No. 1952..	Rabbit No. 1903	22/ 8/57	30·0 iv..	7	22/ 8/57	1·5	Very good	80	0	Highest temperature recorded 106·2 °F
					5/ 9/57	3·0	Very good	30	50	No further clinical symptoms
					20/ 9/57	1·5	0	0	75	
					28/ 9/57	2·0	0	0	90	
					7/10/57	2·0	0	0	90	
					14/10/57	2·0	0	0	No sperms	
					23/10/57	2·0	0	0	0	
					28/10/57	0·5	0	0	0	
					11/11/57	0·75	0	30	75	
					19/11/57	1·0	Fair.....	30	35	
					2/12/57	2·0	Fair.....	60	35	
					6/ 1/58	2·0	Very good	80	0	
	Rabbit No. 1977	23/ 1/58*	30·0 iv..	—	7/ 2/58	1·0	Good...	78	0	
					18/ 2/58	3·0	Good...	70	0	
	Rabbit No. 2338	2/ 4/59*	30·0 iv..	—	—	—	—	—	—	No clinical reaction

* — Immunity Test.

TABLE H

Response of artificially B. besnoiti *infected rabbits to treatment during period of reaction*

Rabbit No.	*Donor No.	Date of Infection	Blood in ml.	Incubation Period in Days	Examination of Blood Smears: Date	Examination of Blood Smears: Parasites	Clinical Symptoms: Oedema	Clinical Symptoms: Max. Temp. F	Duration of Disease in Days	Treatment: Drug	Treatment: Dose per Kg. Body Weight	Treatment: Method	Severity of Disease	Remarks
5	67	20 4 54	3·0 ip...	7	29 4 54 30 4 54	· ·	·	105·4	4	Phenergan..... Sulfamerazine..	0·5 ml. daily..... 500 mgm. followed by 200 mgm. daily for 2 days	sc. per os	· ·	Died
6	67	20 4 54	3·0 ip...	10	1 5 54	·	·	105·0	5	Phenergan..... Sulfamerazine..	0·5 ml. daily..... 500 mgm. followed by 200 mgm. for 5 days	sc. per os	· ·	Died
54	39	19 3 54	2·5 ip...	8	29 3 54 31 3 54 1 4 54	· · · ·		106·2	4	Phenergan..... Sulfamerazine..	0·75 ml. daily..... 600 mgm. followed by 300 mgm. daily for 2 days	sc. per os	· ·	Died
55	39	19 3 54	2·5 ip...	12	31 3 54 1 4 54	· ·	·	105·4	8	Phenergan..... Sulfamerazine..	0·75 ml. daily..... 600 mgm. followed by 300 mgm. daily for 2 days	sc. per os	· ·	Died
57	49	26 3 54	2·5 ip...	8	7 4 54 8 4 54 9 4 54	· · ·	·	105·8	7	Sulfamerazine..	300 mgm. daily for 4 days	per os	· ·	Recovered
	96 and 98	†19 5 54	5·0 ip...											Immune
58	54	31 3 54	2·0 ip...	6	7 4 54	·	·	106·0	4	Sulfamerazine..	400 mgm. for 1 day ...	per os	·	Died
59	54	31 3 54	2·0 ip...	6	9 4 54	·	·	106·6	5	Sulfamerazine..	400 mgm. daily for 2 days	per os	· ·	Died
60	54	31 3 54	2·0 ip...	7	10 4 54	·	·	106·8	3	Sulfamerazine..	400 mgm. for 1 day....	per os	· ·	Died
61	54	31 3 54	2·0 ip...	7	9 4 54 10 4 54	·	·	105·4	4	Sulfamerazine..	400 mgm. daily for 2 days	per os	· ·	Died
62	54	31 3 54	2·0 ip...	8	9 4 54	·	·	106·4	7	Sulfamerazine..	400 mgm. daily for 4 days	per os	· ·	Recovered
	96 and 98	†19 5 54	5·0 ip...					102·8						Immune
64	56	7 4 54	2·5 ip...	6	14 4 54	·	·	106·2	7	Sulfamerazine..	400 mgm. for 1 day.....	per os	·	Recovered
65	56	7 4 54	2·5 ip...	6	14 4 54	·	· ·	106·2	25	Sulfamerazine..	400 mgm. daily for 4 days	per os	· ·	Died
75	67	20 4 54	3·0 ip...	9	30 4 54 1 5 54	· ·	·	105·4	4	Sulfamerazine..	400 mgm. daily for 4 days	per os	· ·	Died
76	67	20 4 54	5·0 ip...	9	1 5 54	·		106·4	7	Sulfamerazine..	400 mgm. daily for 5 days	per os	· ·	Recovered
77	67	20 4 54	3·0 ip...	8				104·8	7	Sulfamerazine..	400 mgm. daily for 5 days	per os	·	Recovered
78	67	20 4 54	3·0 ip...	9				105·0	6	Sulfamerazine..	400 mgm. daily for 5 days	per os	·	Recovered
79	67	20 4 54	3·0 ip...	10	30 4 54 1 5 54	· ·		105·8	5	Sulfamerazine..	400 mgm. daily for 5 days	per os	·	Died
84	80	29 4 54	2·5 ip...	8	10 5 54	·		104·0	4	Sulfamerazine..	400 mgm. for 1 day.....	per os	· ·	Died
85	80	29 4 54	2·5 ip...	11	13 5 54 14 5 54	· ·		106·4	6	Sulfamerazine..	400 mgm. daily for 3 days	per os	· ·	Died

* Rabbit unless otherwise stated † Immunity test

TABLE H (*continued*)

Rabbit No.	*Donor No.	Date of Infection	Blood in ml.	Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Treatment			Severity of Disease	Remarks
					Date	Parasites	Oedema	Max. Temp. °F		Drug	Dose per Kg. Body Weight	Method		
39	24	4/ 3/54	2·5 ip..	13	19/ 3/54 20/ 3/54	+ ++	+	107·0	3	Sulfamezathine (16% solution)	1·0 ml. daily for 2 days	iv.	++	Died
41	30	6/ 3/54	2·5 ip..	11	17/ 3/54 18/ 3/54	+ +	+	104·8	4	Sulfamezathine.	1·0 ml. daily for 2 days	iv.	++	Died
42	30	6/ 3/54	10·0 sc..	8	16/ 3/54 17/ 3/54	+ +	+	106·2	7	Sulfamezathine.	1·0 ml. daily for 3 days	iv.	++	Recovered
	†96 and 98	19/ 5/54	5·0 ip..	—	—	—	—	103·2	—	—	—	—	—	Immune
43	5870 ox	6/ 3/54	3·0 ip..	12	22/ 3/54	++	+	105·6	4	Sulfamezathine.	1·5 ml. for 1 day.....	iv.	++	Died
46	28	8/ 3/54	3·0 ip..	5	16/ 3/54 17/ 3/54 18/ 3/54	++ +++ ++++	+++	106·6	6	Sulfamezathine.	1·5 ml. daily for 2 days	iv.	+++	Died
47	28	8/ 3/54	6·0 sc..	9	20/ 3/54	+	—	106·0	7	Sulfamezathine.	1·5 ml. for 1 day.....	iv.	++	Recovered
48	5870 ox	10/ 3/54	3·0 ip..	12	22/ 3/54 23/ 3/54 24/ 3/54	+ ++ +	+	106·2	9	Sulfamezathine.	1·5 ml. daily for 5 days	iv.	++	Died
49	5870 ox	10/ 3/54	3·0 ip..	13	26/ 3/54 27/ 3/54 29/ 3/54	+	+	106·0	7	Sulfamezathine.	1·5 ml. daily for 5 days	iv.	++	Died
50	35	12/ 3/54	2·5 ip..	7	22/ 3/54 23/ 3/54	+ ++	+	106·6	5	Sulfamezathine.	1·5 ml. daily for 3 days	iv.	++	Died
52	40	12/ 3/54	2·5 ip..	7	23/ 3/54 24/ 3/54	+ +	+	106·4	6	Sulfamezathine.	1·5 ml. daily for 2 days	iv.	++	Died
86	80	29/ 4/54	2·5 ip..	6	—	—	—	105·6	15	Sulfamezathine.	1·5 ml. daily for 3 days	iv.	+	Died
87	80	29/ 4/54	2·5 ip..	6	—	—	—	106·6	6	Sulfamezathine.	1·5 ml. for 1 day.....	iv.	+	Died
19	1	16/ 2/54	2·5 ip..	6	24/ 2/54 25/ 2/54 26/ 2/54 27/ 2/54	+ + ++ ++	++	105·2	13	Aureomycin.... (1% solution)	10·0 mgm. daily for 5 days	iv.	+++	Died
25	2	18/ 2/54	6·0 ip..	5	26/ 2/54 27/ 2/54 28/ 2/54 1/ 3/54	+++ +++ +++ +++	+	106·2	7	Aureomycin....	10·0 mgm. daily for 3 days	iv.	++	Died
31	18	25/ 2/54	3·0 sc..	11	9/ 3/54	+	+	105·0	3	Aureomycin....	10·0 mgm. daily for 3 days	iv.	++	Died
33	19	27/ 2/54	3·0 ip..	9	8/ 3/54	+	++	105·6	3	Aureomycin....	10·0 mgm. daily for 2 days	iv.	+++	Died

* Rabbit unless otherwise stated † = Immunity test

TABLE H (*continued*)

Rabbit No.	*Donor No.	Date of Infection	Blood in ml.	Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Treatment			Severity of Disease	Remarks
					Date	Parasites	Oedema	Max. Temp. °F		Drug	Dose per Kg. Body Weight	Method		
92	89	8/ 5/54	2·5 ip..	6	18/ 5/54	+	+	106·2	10	Phenergan.....	0·5 ml. daily.....	im.	++	Died
										Achromycin.... (1% solution)	10·0 mgm. daily for 4 days	iv.		
93	83	10/ 5/54	2·5 ip..	6	19/ 5/54	+	+++	105·4	6	Phenergan.....	0·5 ml. daily.....	im.	++	Died
										Achromycin....	10·0 mgm. daily for 3 days	iv.		
95	83	10/ 5/54	2·5 ip..	7	22/ 5/54	++	—	105·0	11	Phenergan.....	0·5 ml. daily.....	im.	++	Died
										Achromycin....	10·0 mgm. daily for 4 days	iv.		
96	83	10/ 5/54	2·5 ip..	7	19/ 5/54	+	—	105·6	52	Phenergan.....	0·5 ml. daily.....	im.	++	Died
										Achromycin....	10·0 mgm. daily for 4 days	iv.		
97	83	10/ 5/54	2·5 ip..	6	—	—	+	104·8	4	Phenergan.....	0·5 ml.....	im.	+	Died
										Achromycin....	10·0 mgm. for one day.	iv.		
98	83	10/ 5/54	2·5 ip..	9	19/ 5/54	+	—	105·2	7	Phenergan.....	0·5 ml. daily.....	im.	++	Recovered
					20/ 5/54	+				Achromycin....	10·0 mgm. daily for 4 days	iv.		
34	19	27/ 2/54	3·0 sc..	12	12/ 3/54	+	+++	105·2	5	Babesin (Hoechst.)	3·0 mgm. for 1 day....	iv.	+++	Died
					13/ 3/54	++								
					14/ 3/54	++								
35	19	27/ 2/54	3·0 sc..	10	12/ 3/54	++	+	105·0	5	Babesin.....	4·0 mgm. for 1 day....	iv.	++	Died
38	24	4/ 3/54	2·5 ip..	7	12/ 3/54	+	++	106·0	7	Gonacrine (5% solution)	0·05 mgm. for 1 day....	iv.	+++	Died
					13/ 3/54	++								
					15/ 3/54	+++								
40	30	6/ 3/54	2·5 ip..	6	12/ 3/54	++	++	105·8	5	Gonacrine.....	0·05 mgm. for 1 day...	iv.	+++	Died
					13/ 3/54	++								
					14/ 3/54	++								
1550	1539 & 1541	16/ 5/56	5·0 ip..	15	6/ 6/56	++	—	105·4	7	Pentamidine (1% solution)	1·5 ml. daily for 2 days	sc.	++	Recovered
					7/ 6/56	++								
1552	1539 & 1541	16/ 5/56	5·0 ip..	14	5/ 6/56	+	+	105·4	15	Pentamidine....	2·0 ml. for 1 day.....	sc.	++	Died
1553	1539 & 1541	16/ 5/56	5·0 ip..	14	7/ 6/56	++		105·0	7	Pentamidine....	2·5 ml. daily for 2 days	sc.	++	Recovered

* Rabbit unless otherwise stated † — Immunity test

TABLE H (*continued*)

Rabbit No.	*Donor No.	Date of Infection	Blood in ml.	Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Treatment			Severity of Disease	Remarks
					Date	Parasites	Oedema	Max. Temp. °F		Drug	Dose per Kg. Body Weight	Method		
80	67	20/ 4/54	3·0 ip..	6	29/ 4/54 30/ 4/54	+ +	+	105·6	5	Phenamidine (5% solution)	0·4 ml. for 1 day.....	sc.	++	Died
81	65	21/ 4/54	3·0 ip..	7	1/ 5/54	+	–	105·8	8	Phenamidine...	0·4 ml. daily for 2 days	sc.	++	Died
82	65	21/ 4/54	3·0 ip..	7	30/ 4/54 1/ 5/54	+	+	106·0	3	Phenamidine...	0·4 ml. for 1 day.....	sc.	++	Died
89	74	29/ 4/54	2·5 ip..	5	8/ 5/54	+	–	106·8	7	Phenamidine...	0·5 ml. daily for 2 days	sc.	+	Recovered
90	74	29/ 4/54	2·5 ip..	5	—	–	–	106·4	10	Phenamidine...	0·5 ml. daily for 2 days	sc.	+	Recovered
88	80	29/ 4/54	2·5 ip..	5	10/ 5/54	+	–	106·0	7	Plasmoquine (16% solution)	0·5 ml. for 1 day.....	iv.	++	Died
1251	79328 (goat)	12/10/54	6·0 ip..	13	27/10/54 28/10/54 29/10/54	+ ++ +++	++	107·0	12	Sodium iodide (10% solution)	2·5 ml. twice at 3 daily intervals	iv.	+++	Died
1252	79328 (goat)	12/10/54	6·0 ip..	9	25/10/54 27/10/54 28/10/54	+ ++ +++	+	106·0	13	Sodium iodide.	2·5 ml. for 1 day.....	iv.	++	Died

* Rabbit unless otherwise stated † =Immunity test

TABLE I

Repeated treatment of 26 rabbits during incubation period, continued in 19 cases also during reaction period

Rabbit No.	Donor No.	Date of Infection 5·0 ml. Blood ip.	Controls Table C	Incubation Period in Days	Examination of Blood Smears: Date	Examination of Blood Smears: Parasites	Clinical Symptoms: Oedema	Clinical Symptoms: Max. Temp. F	Duration of Disease in Days	Treatment: Drug	Treatment: Dose per Kg. Body Weight	Treatment: Days after Infection	Treatment: Method	Severity of Reaction	Result
1566	1556	30 5 56	Gen. 76	16	19 6 57			106·0	8	Trypan blue (1% solution)	4·0 mgm.	2, 4, 6, 8, 10, 12, 14, 16	iv.		Recovered
1567	1556	30 5 56	Gen. 76	8	10 6 57			105·4	7	Pentamidine (1% solution)	0·5 ml.	2, 4, 6, 8, 10, 12	sc.		Recovered
1568	1556	30 5 56	Gen. 76	10	13 6 57			106·0	8	Pentamidine	1·0 ml.	2, 4, 6, 8, 10, 12, 14, 16, 18, 20, 22	sc.		Recovered
1569	1556	30 5 56	Gen. 76	10	13 6 57			105·0	9	Mycostatin Phenergan	500,000 units 0·75 ml.	2, 4, 6, 8, 10, 12	per os im.		Died
1583	1563	15 6 56	Gen. 77	10				107·0	5	Phenamidine (5% solution)	0·6 ml.	2, 4, 6, 8, 11, 13, 15	sc.		Died
1584	1563	15 6 56	Gen. 77	7	2 7 56			105·4	11	Anthiomaline	0·04 ml.	3, 6, 10, 13	im.		Died
1630	1614	20 8 56	Gen. 82	7	31 8 56			105·0	7	Pamaquin	0·2 ml.	2, 4, 6, 8	im.		Recovered
1631	1614	20 8 56	Gen. 82	9	4 9 56			106·2	8	Aralen	0·4 ml.	2, 4, 6, 8	im.		Recovered
1632	1614	20 8 56	Gen. 82	77				106·6	5	Aralen	100 mgm.	2, 4, 6, 8	per os		Died
1684	1668	8 10 56	Gen. 86	9	20 10 56			104·4	5	Mistamycin	400,000 units	2, 4, 6, 8	im.		Died
1686	1668	8 10 56	Gen. 86	8	20 10 56			104·8	6	Mistamycin Phenergan	400,000 units 0·75 ml.	2, 4, 6, 8	im. im.		Died
1685	1668 & 1696	8 10 56 *9 11 56	Gen. 86	15				105·0 103·0	7	Terramycin	10·0 mgm.	2, 4, 6, 8	iv.		Recovered *Immune*
1707	1695	9 11 56	Gen. 89	14	28 11 56			103·8	5	Terramycin	20·0 mgm.	3, 4, 5, 6, 7, 8	iv.		Died
1724	1714	1 12 56	Gen. 91	9	13 12 56			105·0	8	Quinacrine	9·0 mgm.	3, 4, 5, 6, 7, 8	per os		Died
1725	1714	1 12 56	Gen. 91	9	14 12 56			105·6	7	Quinacrine	9·0 mgm.	3, 4, 5, 6, 7, 8	per os		Recovered
1726	1714	1 12 56	Gen. 91	8	13 12 56			105·8	7	Quinacrine	9·0 mgm.	3, 4, 5, 6, 7, 8	per os		Recovered

* Immunity test

Parasites: rare, *infrequent*, frequent

Oedema: mild, *severe*, very severe

Severity: mild, severe, very severe

TABLE I (*continued*)

Rabbit No.	Donor No.	Date of Infection 5·0 ml. Blood ip.	Controls Table C	Incubation Period in Days	Examination of Blood Smears		Clinical Symptoms		Duration of Disease in Days	Treatment				Severity of Reaction	Result
					Date	Parasites	Oedema	Max. Temp. °F		Drug	Dose per Kg. Body Weight	Days after Infection	Method		
1912	1907	22/ 8/57	Gen. 115	7	4/ 9/57	++	−	107·0	9	Spirotrypan....	0·2 ml.....	2, 4, 6, 8, 10, 12	im.	+++	Died
1913	1907	22/ 8/57	Gen. 115	8	10/ 9/57	+	−	105·0	7	Spirotrypan....	0·4 ml.....	2, 4, 5, 8, 10, 12	im.	+	Recovered
1914	1907	22/ 8/57	Gen. 115	7	8/ 9/57	+	−	105·0	7	Spirotrypan....	0·4 ml.....	2, 4, 6, 8, 10, 12	im.	++	Recovered
1915	1907	22/ 8/57	Gen. 115	8	—	−	+	105·6	10	Spirotrypan....	0·6 ml.....	2, 4, 6, 8, 10, 12	im.	++	Died
1928	1920	21/ 9/57	Gen. 117	7	2/10/57	+	−	106·0	7	Formalin (1% solution)	0·4 ml.....	2, 4, 6, 8	iv.	++	Recovered
1929	1920	21/ 9/57	Gen. 117	8	4/10/57	+	−	105·4	7	Formalin (1% solution)	0·4 ml.....	2, 4, 6, 8	iv.	++	Recovered
1930	1920	21/ 9/57	Gen. 117	6	1/10/57	+	+	104·8	7	Formalin.....	0·6 ml.....	2, 4, 6, 8	iv.	++	Recovered
1931	1920	21/ 9/57	Gen. 117	5	2/10/57	+	−	105·0	7	Formalin.....	0·8 ml.....	2, 4, 6, 8	iv.	++	Recovered
1950	1944	2/11/57	Gen. 121	8	13/11/57	+	−	105·0	8	Tubomel (INH) P.A.S.....	20·0 mgm... 200 mgm....	3, 4, 5, 6, 7, 8	per os	++	Recovered
1951	1944	2/11/57	Gen. 121	9	13/11/57 14/11/57	+ +	+	105·4	8	Tubomel (INH) P.A.S.....	20·0 mgm... 200 mgm....	3, 4, 5, 6, 7, 8	per os	++	Recovered

Parasites + = rare, ++ = infrequent, +++ = frequent

* Immunity test

Oedema + = mild, ++ = severe, +++ = very severe

Severity + = mild, ++ = severe, +++ = very severe