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THE ARTIFICIAL TRANSMISSION OF *BESNOITIA BESNOITI* (MAROTEL, 1912) FROM CHRONICALLY INFECTED TO SUSCEPTIBLE CATTLE AND RABBITS

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INTRODUCTION

Besnoitia besnoiti was first described by Besnoit & Robin (1912), who observed the thick-walled parasitic cysts in the skin of a chronically infected cow in Southern France. The crescentic organisms within the cysts, which they called spores, will be referred to as cyst forms or cyst organisms in this discussion, in contradistinction to proliferative forms which are encountered in macrophages or lying free during the acute stage of the disease.

It is well known that cattle which survive the acute phase of besnoitiosis remain carriers of large numbers of cysts for life (Pols, 1960). Hence they should be able to serve as ideal reservoirs of the disease. However, previous attempts by various investigators to transmit cyst organisms to a variety of animals have apparently all been unsuccessful. Besnoit & Robin (1912), who used a suspension of cyst-bearing skin as inoculum, failed to transmit the disease to cattle by the subcutaneous and intramammary routes, and by rubbing the material into the scarified skin. Equally unsuccessful were their attempts to transmit besnoitiosis to rabbits, white rats and mice by feeding them on bovine skin harbouring numerous cysts. Franco & Borges (1916) also failed to infect rats and mice by administering infected tissues from chronically infected cattle by the oral and subcutaneous routes.

The first successful transmission of bovine besnoitiosis was achieved by Cuillé, Chelle & Berlureau (1936). They reproduced the disease by injecting cattle intravenously with large volumes of blood collected from a natural case during the initial stages of the thermal reaction. As they could not demonstrate the infectious agent microscopically in the blood of the donor at the time of subinoculation, nor in that of the recipient during the ensuing febrile reaction, they suggested that *B. besnoiti* was either extremely rare in the blood, or that it occurred in a form different from that later observed in the skin. Cuillé & Chelle (1937a) also tried to transmit besnoitiosis to cattle by administering a suspension of skin cysts by the oral, subcutaneous, intracutaneous or intramammary routes, but there was no evidence of successful transmission. They, therefore, concluded that under experimental conditions bovine besnoitiosis could only be transmitted provided blood collected during the thermal reaction was used as inoculum.

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Pols (1954a) not only confirmed the work of Cuillé *et al.* (1936), but also showed that *B. besnoiti* proliferative forms (which he called trophozoites) are demonstrable microscopically, lying free or intracellularly in monocytes in blood and lymph node smears made during the initial febrile phase of besnoitiosis. He also established that rabbits were highly susceptible to infection with proliferative forms. Pols (1960) likewise tried to transmit the disease to cattle by subcutaneous implantation of connective tissue heavily infected with *Besnoitia* cysts and by injecting a skin suspension containing numerous cyst organisms subcutaneously and intravenously. There was, however, no evidence of successful transmission.

The first indication that cyst organisms of *Besnoitia* were infective was presented by Frenkel (1953) while conducting investigations on *Besnoitia jellisoni* Frenkel, 1953, harboured by the white-footed mouse [*Peromyscus maniculatus artemesiae* (Wagner)]. He showed that intraperitoneal injection of cyst organisms of *B. jellisoni* into white mice, hamsters, rats, new-born guinea pigs, chick embryos, *Citellus* sp., *Callospermophilus* sp. and *Peromyscus leucopus noveboracensis* (Fischer) resulted in an acute infection, much like that of toxoplasmosis. The disease terminated fatally in four to twelve days. Subinoculation of cyst forms into the natural host, *Microtus modestus* (Baird), or some of the abovementioned laboratory animals, if they were treated with sulphonamides or aureomycin, resulted in the formation of thick-walled spherical cysts.

Consideration of these successful transmission experiments, and the strikingly similar morphological and biological characteristics of *B. jellisoni* and *B. besnoiti*, cast doubt on the conclusions arrived at by Cuillé *et al.* (1937a) and Pols (1960). It became apparent that critical experiments would have to be conducted before convincing conclusions could be drawn about the non-infectivity of cyst organisms of *B. besnoiti*. This decision was fully justified when it was found that neither Cuillé *et al.* (1937a) nor Pols (1960) had taken the possibility of cattle developing an inapparent infection into consideration, and that the highly susceptible rabbit had not been employed for these studies. Not only does this animal develop pronounced clinical symptoms after infection with proliferative forms, but careful examination of blood and tissue smears reveals free and intracytoplasmic parasites in monocytes (Pols, 1954 a, b).

The experiments which are to be described comprise attempts to transmit besnoitiosis from clinically apparent, chronically infected cattle to susceptible rabbits and cattle by inoculation with suspensions of tissues harbouring numerous cysts, and hence also cyst organisms.

Materials and Methods

Sources of cyst-bearing tissues

Tissues harbouring numerous cysts of *B. besnoiti* were obtained from cattle showing typical symptoms of chronic besnoitiosis which they had contracted naturally in various districts of the Transvaal bushveld and north-western Cape Province (Table 1). Citrated blood was drawn concurrently from the majority of these animals and inoculated into rabbits by the intraperitoneal or intravenous routes to exclude the possibility of organisms being present in the circulating blood. In an attempt to avoid contaminating the blood with organisms from cysts ruptured accidentally, the following precautions were taken: Blood was allowed to run copiously through the needle inserted into the jugular vein before it was collected; in one case the jugular vein was exposed surgically and the site drenched with absolute alcohol before the needle was inserted; in other cases blood was collected from the heart immediately after slaughter.

TABLE 1.—*History of donors*

Bovine No.	Breed	Sex	District of origin	Date of purchase	Date used as donor
4197.....	Hereford.....	bull	Potgietersrus.	9 Sep. 1949	10 Jan. 1958
4093.....	Friesian-cross	cow	Potgietersrus.	19 Mar. 1949	7 Feb. 1958
Ox (Lamprechts).	Afrikaner....	ox	Thabazimbi..	—	12 Jun. 1959
130.....	Afrikaner....	bull	Vryburg.....	18 Jun. 1959	2 Nov. 1961
688.....	Afrikaner....	bull	Groot Marico	20 May 1960	2 Nov. 1961
Heifer (Faculty)*	Afrikaner....	heifer	Brits.....	—	4 Aug. 1958
3865.....	Friesian-cross	ox	Rustenburg..	12 Jan. 1965	11 Feb. 1965
895.....	Afrikaner....	bull	Zoutpansberg	19 Oct. 1960	26 Jan. 1966

* Blood only was injected into rabbits from this case (see Table 2). This was done in order to determine whether the free-lying besnoitias found in a blood smear were evidence of parasitaemia or the result of accidental rupture of a cyst whilst preparing the smears.

Preparation of infective inoculum

In most cases this was prepared by cutting cyst-bearing tissues into small pieces and grinding them in a mortar after normal saline or Hanks' solution had been added. The former contained 500 units penicillin and 500 micrograms streptomycin per ml, and the latter 200 units penicillin, 200 micrograms streptomycin and 2 micrograms fungizone per ml. These antibiotics have been used previously in similar and higher concentrations without any obvious deleterious effect (Bigalke, 1962). The resulting suspensions were then strained through two layers of gauze to remove the larger particles. This procedure inevitably removed many cyst organisms too. To obviate this loss, in mild cases particularly, cysts were dissected out of the dermis to obtain them reasonably free from host tissues before crushing them in a mortar as described previously.

The approximate number of cyst forms in the inoculum was determined by counting them in a haemocytometer at suitable dilutions in two instances (Ox 3865 and Bull 895); in all cases smears made from the inoculum were stained with Giemsa and examined for parasites, which were invariably present. The suspensions were then injected into rabbits or cattle.

Injection of rabbits with infective inoculum

Full-grown rabbits, of which the majority were males bred at this Institute, were injected subcutaneously or intraperitoneally with infective inoculum (Table 2). The rabbits were housed singly in wire cages; their temperatures were recorded and blood smears prepared daily. In some cases, where proliferative organisms could not be found, smears were made from the testis to confirm their suspected presence (Pols, 1960). Usually the newly isolated *B. besnoiti* strains were passaged in rabbits for one or more generations by subinoculation of blood at the height of the febrile reaction.

TABLE 2.—*Transmission to rabbits*

Bovine donor	Rabit recipient	Inoculum	Route	Dose (ml)	Date of infection	Result				Immunity Test			
						Incubation period (days)	Proliferative forms	Cysts	Symptoms and fate	Time after infection (days)	Strain	Passage level	Result
4197.....	RH	nasal mucosa	i.p.	10	10 Jan. 58	10	+	n.e.	−(D)	—	—	—	—
	RB	fascia	i.p.	3	10 Jan. 58	12	+	n.e.	+(K)	—	—	—	—
4093.....	2021	skin	s.c.	5	27 Feb. 58	10	+	+	+(S)	46	Fuls	135	No reaction
	2022	skin	s.c.	5	27 Feb. 58	10	+	+	+(S)	46	Fuls	135	No reaction
	2023	skin	s.c.	5	27 Feb. 58	10	+	+	+(S)	46	Fuls	135	No reaction
	2024	skin	s.c.	5	27 Feb. 58	13	+	+	+(S)	46	Fuls	135	No reaction
	2042	skin	i.p.	7	27 Mar. 58	16	+	n.e.	+(K)	—	—	—	—
	2043	skin	i.p.	7	27 Mar. 58	14	+	n.e.	+(K)	—	—	—	—
	2044	skin	i.p.	7	27 Mar. 58	20	+	n.e.	+(K)	—	—	—	—
	2177	skin	s.c.	8	10 Aug. 58	9	+	+	+(S)	—	—	—	—
	2178	skin	s.c.	8	10 Aug. 58	10	+	+	+(S)	—	—	—	—
	2181	skin	s.c.	8	10 Aug. 58	13	+	n.e.	+(D)	—	—	—	—
	2185	skin	i.p.	10	10 Aug. 58	9	+	+	+(S)	—	—	—	—
	2165	blood	i.v.	10	27 Aug. 58	—	—	n.e.	−(S)	—	—	—	—
	2166	blood	i.v.	10	27 Aug. 58	—	—	n.e.	−(S)	—	—	—	—
Ox..... (Lamprechts)	2420	skin	s.c.	5	12 Jun. 59	10	+	—	+(S)	95	Fuls	181	No reaction
	2416	blood	i.p.	10	12 Jun. 59	—	—	n.e.	−(S)	27	Fuls	175	Fully susceptible

TABLE 2.—*Transmission to rabbits* (continued)

Bovine donor	Rabit recipient	Inoculum	Route	Dose (ml)	Date of infection	Result				Immunity Test			
						Incubation period (days)	Proliferative forms	Cysts	Symptoms and fate	Time after infection (days)	Strain	Passage level	Result
Heifer.....	2169	blood	i.p.	25	4 Aug 58	—	—	n.e.	−(D)	—	—	—	—
(Faculty).....	2170	blood	i.p.	25	4 Aug. 58	—	—	n.e.	−(S)	75	Fuls	153	Fully susceptible
3865.....	4333	skin	s.c.	8*	12 Feb. 65	11	+	n.e.	+(D)	—	—	—	—
	4334	skin	s.c.	8*	12 Feb. 65	11	+	n.e.	+(S)	—	—	—	—
	4325	blood	i.p.	20	11 Feb. 65	—	—	n.e.	−(S)	44	Schoeman	147	Fully susceptible
	4326	blood	i.p.	20	11 Feb. 65	—	—	n.e.	−(S)	60	do.	148	Fully susceptible
	4327	blood	i.p.	20	11 Feb. 65	—	—	n.e.	−(S)	44	do.	147	Fully susceptible
	4328	blood	i.p.	20	12 Feb. 65	—	—	n.e.	−(S)	43	do.	147	Fully susceptible
	4329	blood	i.p.	20	12 Feb. 65	—	—	n.e.	−(S)	43	do.	147	Fully susceptible
	4330	blood	i.p.	20	12 Feb. 65	—	—	n.e.	−(S)	43	do.	147	Fully susceptible
	4339	blood	i.p.	20	12 Feb. 65	—	—	n.e.	−(S)	43	do.	147	Fully susceptible
895.....	4692	skin	s.c.	5†	27 Jan. 66	?	—	n.e.	−(S)	26	Fuls	499	No reaction
	4693	skin	s.c.	2·5†	27 Jan. 66	12	+	n.e.	+(K)	—	—	—	—
	4694	skin	s.c.	2·5	27 Jan. 66	11	+	n.e.	+(K)	—	—	—	—
	4684	blood	i.p.	20	26 Jan. 66	—	—	n.e.	−(S)	27	Fuls	499	Fully susceptible
	4685	blood	i.p.	20	26 Jan. 66	—	—	n.e.	−(S)	27	Fuls	499	Fully susceptible
	4686	blood	i.p.	20	26 Jan. 66	—	—	n.e.	−(S)	27	Fuls	499	Fully susceptible

Note: i.p. = intraperitoneal s.c. = subcutaneous i.v. = intravenous n.e. = not examined D = died S = survived
K = killed * = Inoculum contained ± 815,000 organisms/ml † = Inoculum contained ± 1,250,000 organisms/ml

A strain isolated from Cow 4093 was injected into a splenectomized Calf 9040 after two rabbit passages. When Calf 9040 became febrile, 500 ml blood was injected into Cow 6719. Two rabbits were injected with blood from this animal to verify that *B. besnoiti* had caused its thermal reaction.

Injection of cattle with infective inoculum

Six grade Herefords bred at this Institute under tick-free conditions were used for this purpose. They varied in age from nine months to four years and were all oxen, with the exception of Heifer 8494. Oxen 183 and 6890 had been splenectomized as calves.

The animals were injected with infective inoculum by various routes as indicated in Table 4. They were housed in stables under tick-free conditions, and their temperatures recorded and blood smears prepared daily. During the ensuing febrile reactions 10 to 30 ml citrated blood from each animal was injected into at least two rabbits on one or more occasions. This procedure was essential because the cattle developed such a mild form of the disease that proliferative forms and cysts were very difficult to find. The management of, and observations on, these rabbits were essentially the same as described previously. Approximately one litre of blood was also transfused into Cow 5407 from Heifer 8494 on the third day of pyrexia.

Examinations for cysts in the experimental animals

Approximately six to eight weeks after the febrile reaction skin specimens were collected from the upper lip and nose of surviving rabbits and the neck of cattle These were sectioned, stained with hematoxylin and eosin and examined for the typical thick-walled cysts of *B. besnoiti*.

The scleral conjunctiva of all the cattle listed in Table 4, except for Ox 6890, was also examined for cysts by the method of Bigalke & Naudé (1962). Since the value of this diagnostic procedure was unknown at the time the bulk of this work was done, not all the cattle were examined. Fortunately Heifer 8494 was still available.

Immunity tests

The immunity of most of the rabbits and cattle was challenged from 28 to 110 days after infection. The " Fuls " and " Schoeman " strains were used for this purpose; these strains had been passaged in rabbits since their isolation from acute cases of bovine besnoitiosis in 1953 (Pols, 1954a) and 1960 (Bigalke & Schutte, 1960) respectively. Infective blood was drawn from parasitaemic rabbits by cardiac puncture. Rabbits were injected by intraperitoneal or subcutaneous, and cattle by the latter and intravenous routes. Susceptible rabbits injected concurrently served as controls for the infectivity of the blood.

Rabbits which had survived infection with the " Fuls " and " Schoeman " strains were similarly challenged with some of the newly isolated strains.

RESULTS

Transmission to rabbits

The salient features are illustrated in Table 2.

The 19 rabbits injected with infective inoculum contracted typical besnoitiosis as described by Pols (1954a, b) for infection with proliferative forms of *B. besnoiti*. The incubation period varied from nine to twenty days (mean = 11·7). Febrile reactions often exceeded 105° F and lasted from three to eight days. Proliferative forms were demonstrable in blood smears of 18 of the rabbits for one to five days during the thermal reaction. Symptoms included oedematous swellings of the scrotum, prepuce, vulva, legs, ears and face, and necrosis of the scrotum and testes (Plate 1) as described by Pols (1954a, b; 1960). Typical thick-walled *Besnoitia* cysts were found in varying numbers in skin sections of seven of the eight survivors examined.

PLATE 1.—Rabbit showing oedema and necrosis of scrotum

Only two of the 19 rabbits died from the disease. One was killed *in extremis*, four were sacrificed for various purposes such as infecting cattle or further rabbits, and 12 survived. The mortality rate (20 per cent) was considerably lower than that recorded by Pols (1954a) for rabbits infected with bovine blood harbouring proliferative forms, i.e. 100 per cent.

It is interesting to note that 13 rabbits injected with infective inoculum died within 24 hours (Table 3). Of these nine had been injected intraperitoneally and four subcutaneously, representing 60 per cent and 25 per cent respectively of the total number inoculated by each route. On the day after injection the majority of the surviving rabbits had body temperatures exceeding 104° F, which quickly returned to normal.

TABLE 3.—*Rabbits dead within 24 hours of receiving infective inoculum*

Bovine donor	Rabbit recipient	Inoculum	Route	Dose (ml)	Date of infection	Date found dead
4093	2179	skin	i.p.	8	10 Sep. 58	11 Sep. 58
	2180	skin	i.p.	8	10 Sep. 58	11 Sep. 58
	2183	skin	i.p.	12	10 Sep. 58	11 Sep. 58
	2184	skin	i.p.	12	10 Sep. 58	11 Sep. 58
	2202	skin	i.p.	18	9 Oct. 58	10 Oct. 58
	2203	skin	i.p.	18	9 Oct. 58	10 Oct. 58
	2204	skin	i.p.	18	9 Oct. 58	10 Oct. 58
Ox (Lamprechts)	2414	skin	s.c.	5	12 Jun. 59	13 Jun. 59
	2415	skin	s.c.	5	12 Jun. 59	13 Jun. 59
3865	4331	skin	i.p.	8*	12 Feb. 65	13 Feb. 65
	4332	skin	i.p.	8*	12 Feb. 65	13 Feb. 65
895	4688	vein	s.c.	5†	26 Jan. 66	27 Jan. 66
	4689	vein	s.c.	5†	26 Jan. 66	27 Jan. 66

Note: * See note Table 2
† Inoculum contained ± 2,800,000 organisms/ml

A strain isolated from Ox (Lamprechts) was taken through six rabbit passages; six of the ten rabbits used died from besnoitiosis. The splenectomized Calf 9040 infected with a strain isolated in rabbits from Cow 4093 developed a mild reaction. The only sign seen after an incubation period of seven days, was a mild bout of fever lasting three days with a peak of 104·4° F. The reaction in Cow 6719 was also mild. The thermal reaction which developed ten days after infection lasted five days and reached a maximum of 105° F. Although no proliferative forms could be found in blood slides, subinoculation into rabbits proved that they had been present in the blood during the reaction. A few cysts were found in skin sections of Cow 6719, but none in Calf 9040.

In spite of being injected in fairly large volumes, blood obtained from five chronically infected cattle was not infective to a total number of 15 rabbits (Table 2). Four of these bovines served as donors of the cyst-bearing tissues used to prepare the infective inoculum, which was invariably infective. There was, therefore, no evidence of parasitaemia in the donors, and hence cyst forms of *B. besnoiti* were the only likely source of infection.

One of the four rabbits that received blood from Bull 895 died from an unknown cause 48 hours later.

Six rabbits which survived infection with cyst organisms were solidly immune when challenged. This also applied to survivors of the " Fuls " and " Schoeman " strains of *B. besnoiti* when challenged with newly isolated strains.

Transmission to cattle

The salient features are illustrated in Table 4.

The five animals injected with infective inoculum all developed mild besnoitiosis. The incubation periods varied from nine to eleven days. The only perceptible symptom was a fever reaction which lasted seven days in Heifer 8494, but was shorter in the other animals. Ox 1098 did not even develop pyrexia, but one rabbit injected with its blood during the period of the expected reaction developed besnoitiosis. This animal was also solidly immune when challenged. Again splenectomy did not appear to influence the susceptibility.

TABLE 4.—*Transmission to cattle*

Bovine donor	Bovine recipient	Inoculum	Route	Dose (ml)	Date of infection	Result						Immunity Test			
						Incubation period (days)	Proliferative forms	Cysts	Duration pyrexia (days)	Max. temp. (°F)	Rabbit inoculation	Time after infect. (days)	Strain	Passage level	Result
4093.....	6890	skin	i.v. & s.c.	40 & 40	10 Apr. 58	11	+	−	5	105·4	1/2 Pos	—	—	—	—
	8494	skin	i.v. & s.c.	20 & 12	17 Jul. 58	11	−	+	7	104·4	2/2 Pos.	110	Fuls	154	No reaction
688.....	193	skin	i.v. & s.c.	10 & 10	2 Nov. 61	9	−	−	3	103	2/2 Pos.	66	Fuls	259	No reaction
688 & 130	9011	skin	i.v. & s.c.	10 & 10	2 Nov. 61	9	−	−	4	104	2/3 Pos.	66	Fuls	259	No reaction
130.....	1098	skin	i.v. & s.c.	10 & 10	2 Nov. 61	?	−	−	−	102	1/2 Pos.	64	Schoeman	48	No reaction

Note: See Table 2 1/2 denotes 1 rabbit reacted out of 2 injected

Proliferative forms were extremely rare in these cattle. A single, extracellular organism was found after a prolonged search in a blood smear from Ox 6890 on the second day of the febrile reaction. One extracellular, and two intracellular parasites were observed in Cow 5407 on the first day of fever. Unfortunately she died from other causes the following day and the complete reaction, which showed promise of being fairly severe, could not be studied. The reaction in Ox 9011 was no different from that shown by the other cattle in spite of having received cyst organisms from two different donors.

Although *B. besnoiti* was reisolated in rabbits from all five cattle, not every rabbit injected from a reacting bovine became infected. This provides further evidence on the sparseness of proliferative forms in these cattle. They were, however, demonstrated in all rabbits that reacted.

A single cyst was found in the scleral conjunctiva of the left eye of Heifer 8494. This was removed by biopsy and the diagnosis verified microscopically. No cysts were seen in the skin sections or the scleral conjunctiva of the other animals.

Four of the bovines were solidly immune when challenged with the " Fuls " and " Schoeman " strains. The longest time-lapse between infection and challenge was 110 days. The fifth animal was not challenged.

Discussion

Proliferative forms of *Besnoitia* are morphologically virtually indistinguishable from those of *Toxoplasma gondii* (Nicolle & Manceaux, 1908), but the cysts of the former are quite typical (Frenkel, 1953; Pols, 1954b). These were found in varying numbers in the dermis of the majority of rabbits that survived the acute stage of the disease, as well as in two of the cattle involved. It is, therefore, reasonable to assume that the proliferative organisms seen in blood smears were those of *B. besnoiti*.

It is also necessary to consider whether transmission was actually due to cyst forms, or perhaps to proliferative forms which happened to be present in the cyst-bearing tissues used to prepare the infective inoculum. The possibility that cysts rupture periodically, releasing their contents into the circulation was postulated by Besnoit & Robin (1912) who demonstrated cysts in the walls of bloodvessels of a cow. McCully, Basson, Van Niekerk & Bigalke (1966) added weight to this hypothesis when they found that cysts were present in the peripheral veins of chronically infected cattle and antelopes in appreciable numbers.

To date, however, no evidence in favour of this theory has been forthcoming. Pols (1954b, 1960) studied the life cycle of *B. besnoiti* in artificially infected rabbits and cattle. By inoculating them with blood containing proliferative forms a generalized infection was produced. The proliferative organisms then invaded histiocytes and the typical thick-walled cysts were formed. During the period of cyst development proliferative forms ceased to be demonstrable in blood smears. Blood was only infective during the " primary " stage of the disease. The claim made by Bennett (1933) that free " spores " (= cyst organisms) give rise to new " colonies " (= cysts) has never been substantiated. Frenkel (1955) found no indication of renewed proliferative activity after rupture of cysts of *B. jellisoni* in extraneural tissues. Schulz & Thorburn (1955) and Schulz (1960) came to the same conclusions in their studies on equine and bovine besnoitiosis.

In the experiments described in this article in no instance was blood from five cattle with chronic besnoitiosis infective for rabbits, whereas cyst-bearing tissues from these animals invariably transmitted the disease. It can, therefore, be concluded that cyst organisms were responsible for the transmission as they were the only possible source of infection.

The possibility of intermittent, very low-level parasitaemia in chronically infected cattle due to cysts rupturing into the circulating blood has not been ruled out entirely by these experiments. Even at the height of the febrile reactions the numbers of proliferative forms in the blood of the artificially infected cattle must have been very moderate. Had the parasitaemia been much lower it would have been very difficult to detect. These results nevertheless indicate that the likelihood of intermittent parasitaemia occurring to such an extent as to be of significance in the transmission of the disease is very remote.

Considering the ease with which besnoitiosis was transmitted with cyst-bearing tissues, it seems strange that previous research workers (*vide supra*- Introduction) recorded failure. The writer is convinced that Besnoit & Robin (1912), Cuillé *et al.* (1937a) and Pols (1960) probably all succeeded in transmitting bovine besnoitiosis artificially from chronic cases to susceptible cattle without being aware of it because of the mildness of the reactions. In the experiments recorded above the only symptom shown by cattle was a thermal reaction. Proliferative organisms were very rare in blood smears and in only two of the animals were cysts found. Use of the highly susceptible rabbit helped to overcome these difficulties. The possibility that antibodies act on the liberated cyst organisms and reduce their infectivity must be kept in mind as an explanation for this phenomenon.

It is interesting to note that subsequent to the conclusion of the bulk of the work recorded here Bigalke & Naudé (1962) and Bigalke (1966) established that the majority of natural cases of besnoitiosis occurring on a number of farms in the enzootic areas of Transvaal were inapparent, showing nothing but a few cysts in the scleral conjuntiva. Pols (1954a, 1960) has commented on the mildness of the disease caused by artificial infection with proliferative forms. It is therefore evident that the majority of cattle that acquire besnoitiosis, be it naturally or artificially, develop a mild form of the disease. The reason for this remains to be determined.

The transmissibility of cyst organisms has led to new lines of thought on the epidemiology of bovine besnoitiosis. On the majority of farms in the enzootic regions acute cases are not seen during the winter and early spring. In order to explain the appearance of fresh cases the following summer the existence of some mammalian and/or arthropod carrier or reservoir has been assumed. Evidence has now been presented that the so-called " recovered ", chronically infected bovine case, harbouring what probably amounts to many millions of cysts filled with hundreds of thousands of viable parasites in the dermis, subcutis, veins, etc., might well constitute such a reservoir. Such cases are quite numerous on farms in the enzootic regions of Transvaal. In this respect it is noteworthy that Cuillé & Chelle (1937b) observed that early and especially " recovered " cases were capable of introducing the disease into certain uninfected premises in France.

Cuillé *et al.* (1937b), Herin (1952) and Pols (1960) suggested that besnoitiosis was arthropod-borne. Whilst the experiments recorded here were in progress it was realized that the cattle used as donors would probably serve as an excellent source of infection for blood-sucking ectoparasites capable of penetrating the fairly thick walls of the cysts. Subsequent investigations disclosed that *Glossina brevipalpis* Newstead,

1910, various tabanids and *Stomoxys calcitrans* (Linnaeus, 1758) could fulfil these requirements, resulting in the mechanical transmission of cyst forms of *B. besnoiti* to to rabbits and cattle (Bigalke, 1960, 1966). Neuman (1962) confirmed that cyst organisms were infective for rabbits and showed that gerbils (*Meriones tristrami shawii*) were also susceptible.

Under the existing experimental conditions there was complete cross-immunity between the various isolates of *B. besnoiti* in both cattle and rabbits. In future, therefore, immunity to a similar challenge can be regarded as an indication of previous infection with *B. besnoiti*. Whether this challenge is similar to that experienced in nature or not, is open to speculation. No attempt was made to determine the duration of the immunity, but it was still solid after three months. Pols (1960) has submitted evidence that immunity persists in cattle for as long as viable cysts are present, which is life-long for all practical purposes. This probably also applies to rabbits. In this respect, as well as in respect of reservoir potential, it is of considerable significance that cyst-bearing tissues were still infective nine years after purchase of two of the cattle used as donors in this investigation. The mere fact that cyst organisms live so long is remarkable in itself.

There was some evidence that cyst forms were toxic to rabbits if injected in large numbers, more so by the intraperitoneal than subcutaneous route. As death occurred within 24 hours irrespective of the route of administration, and that antibiotics were present in the inoculum, it appears unlikely that bacteria were responsible. This is in accordance with observations made by Besnoit & Robin (1912) who found that a glycerinated extract of affected tissues was highly toxic for rabbits, but not for guinea pigs and rats. Obviously more precise investigations on the possible toxicity of *B. besnoiti* are necessary before final conclusions can be drawn.

Summary

The artificial transmission of besnoitiosis from chronically infected cattle to rabbits and cattle is recorded. The infective inoculum consisted of a suspension of heavily parasitzed cyst-bearing skin and other tissues in saline or Hanks' solution. Large numbers of cyst organisms were present in the inoculum which were apparently responsible for the transmission. Blood from the chronically infected cattle used as donors of the cyst-bearing tissues was not infective.

Cattle were, in turn, infected with blood obtained from the rabbits when they reacted to infection with cyst organisms, thus fulfilling Koch's postulates.

Rabbits developed typical symptoms of besnoitiosis as described for infection with proliferative organisms. Cattle developed a mild form of the disease as described for the majority of animals infected with proliferative forms. The reason for this is obscure, but it is pointed out that the results seen after artificial infection are in agreement with observations made in nature.

On the strength of these findings it is suggested that clinically apparent, chronically infected cattle probably serve as reservoirs of bovine besnoitiosis, and blood-sucking arthropods act as mechanical vectors.

The six strains of *B. besnoiti* isolated in this experiment were immunogenically indistinguishable from two strains isolated previously.

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