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VASCULAR LESIONS IN RABBITS INFECTED WITH *TRYPANOSOMA (TRYPANOZOON) BRUCEI*

BY

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When a rabbit is inoculated with *Trypanosoma* (*Trypanozoon*) *brucei* usually only a few parasites appear in the circulating blood. Nevertheless, 3 to 5 weeks after inoculation the animal becomes listless, the fur staring, and the ears pale, thick and heavy. The tissues around the eyes are swollen, and scabby necrotic lesions appear in the skin of the face, ears and scrotum. The animal dies several weeks after inoculation and the cause of death is unknown.

This paper describes some observations on the circulation in the ears and muscles of normal and infected rabbits and an attempt is made to explain the vascular changes which accompany chronic trypanosome infection.

METHODS

Infection of rabbits

Trypanosomes (*T.*(*T.*) *brucei, strain Tororo 427*) were passaged in mice. The blood of an infected mouse was diluted in phosphate-buffered saline (pH 7.8) and then injected subcutaneously into male New Zealand white rabbits weighing 2.5–3.5 kg. Each rabbit received a volume of blood containing 10^7 trypanosomes and the animals were housed at 23° C.

Anaesthesia

Between 4 and 5 weeks after inoculation with strain 427, when the animals showed signs of the infection, they were anaesthetized with halothane. A tracheal tube was inserted and anaesthesia maintained with N_2O (20%), O_2 (80%) and halothane (0.5–2.5%). The flow rate was 2.5 l./min. A few of the early experiments were carried out under intravenous pentobarbitone anaesthesia.

Angiography

Contrast medium and drugs were introduced into the arterial system of the rabbit's ear through a plastic cannula tied into the right common carotid, the right mandibular or the right subclavian artery. A cannula passed caudally through the left carotid artery into the aortic arch was also tried. The contrast medium, sodium iothalamate (May & Baker) or sodium metrizoate (Glaxo), was injected rapidly through the cannula using either a manually operated syringe or a Cordis injector (pressure: 90 lb./sq. in.). The quantity of contrast medium required depended on the closeness of the cannula to the auricular artery, 2 ml. being sufficient for injection into the mandibular, 3 ml. for injection into the carotid and 8 ml. for injection into the subclavian artery. Radiographs of the ear were taken using a 0.1 mm focus X-ray tube (Rank Medical). Single pictures were usually taken on completion of the injection using industrial film (Gevaert Structurix) but in some experiments eight pictures were taken at intervals of 0.5 sec using a Schonander Serial changer and standard X-ray film (Ilford).

Drugs were given through the arterial cannula and washed in with 1 ml. saline, usually 1 min before the contrast medium. Arterial diameters were measured on the processed films with a graticule.

The following drugs were used: adrenaline hydrochloride (British Drug Houses); hexamethonium bromide (May & Baker); phenoxybenzamine (Smith Kline & French); atropine sulphate (British Drug Houses); bradykinin BRS-640 (Sandoz); histamine acid phosphate (British Drug Houses); and mepyramine maleate (May & Baker). All doses stated refer to the bases.

Twenty normal and twenty-four infected rabbits were used in these experiments.

Blood vessels in cremaster muscles

Rabbits which had been inoculated with *T.* (*T.*) *brucei 427* about 4 weeks previously were injected intravenously with 2–4 ml. of India ink (Pelikan C11/1431; Gunter Wagner) diluted to 1 in 4 with saline. One hour later the animals were killed, the cremaster muscles dissected out, fixed in 10% formol saline, cleared and mounted on glass slides (Majno, Palade & Schoefl, 1961). It was found that a more transparent preparation was obtained if, after fixation, the muscles were dehydrated in increasing concentrations of ethanol, cleared for 24 hr in methyl benzoate and mounted in DePex (Gurr). Part of the muscle was embedded in paraffin and sectioned. Studies were made on four normal and sixteen infected rabbits.

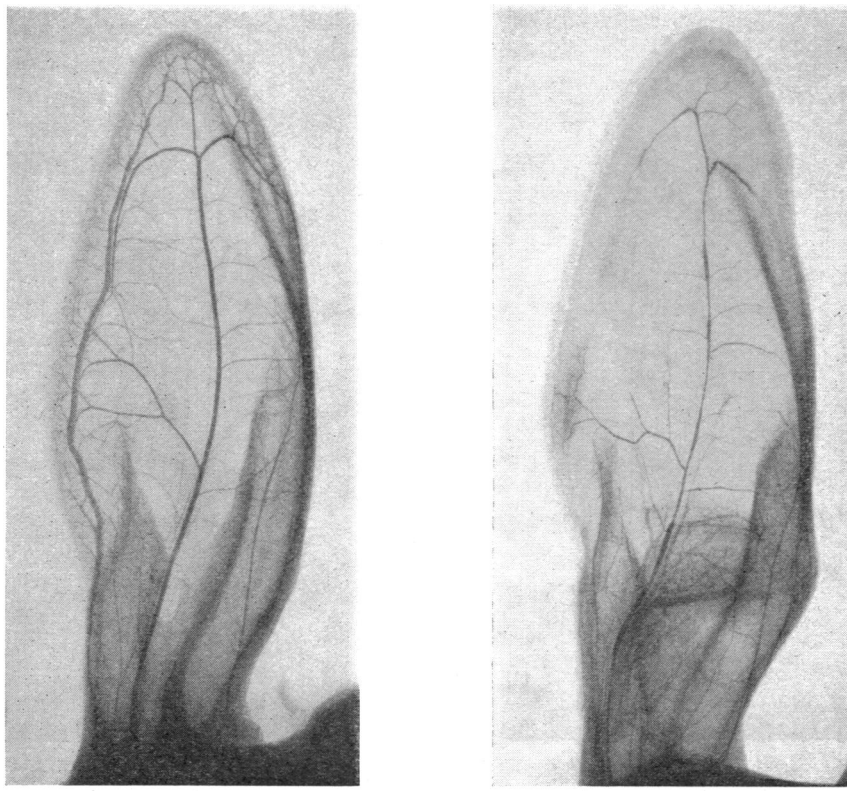
RESULTS

Infected rabbits were more sensitive than normal ones to anaesthetics. Pentobarbitone was unpredictable in its effectiveness and its use was abandoned because respiratory failure frequently occurred in the infected animals. Halothane was easier to control; for maintenance of anaesthesia infected rabbits usually needed 0.5–1.5% halothane whereas normal animals required 1.5–2.5% halothane.

Blood vessels of the ear

Angiography of the ear of a normal rabbit showed a uniformly wide central artery. The diameter was approximately 1 mm and almost constant from the base of the ear to a point, 2–3 cm from the tip, at which the ultimate branching occurred. Along its course branches formed arcades which anastomosed with the marginal veins (Fig. 1a). The central artery of the ear of infected rabbits was narrow (0.3–0.8 mm) and only traces of contrast medium were seen in the parts of the vessel between the origins of lateral branches (Fig. 1b). The arcades were narrow, the passage of contrast medium was slower than in the normal ear and, after several injections had been given, opaque patches often remained in the tissues. A similar, but less irregular picture could be simulated in the normal ear by the injection of adrenaline (3 μg/kg). Hexamethonium (0.2 mg/kg) or phenoxybenzamine (1 mg/kg) caused immediate widening to normal diameter of the vessels of infected rabbits, showing that the narrowing observed in the angiogram was a result of constriction mediated by adrenergic mechanisms (Fig. 2). Atropine (0.5 mg/kg) also caused widening, confirming the observation of Holton & Rand (1962) and others, that vasoconstriction in the rabbit ear is partly mediated by cholinergic mechanisms.

Arterial constriction was observed in the ears of all the infected animals examined: it was most marked in those that had been infected the longest.



a b

Fig. 1. Angiograms of the ears of (a) a normal rabbit and (b) a rabbit infected 4 weeks previously with *T.(T.) brucei*. In the normal animal the central artery is wide and uniform and the arcades are clearly visible. In the infected animal the central artery is narrowed and some of the arcades are barely visible. (×0.75.)

Synthetic bradykinin (0.5 μg/kg) had little effect on the arteries of the normal ear but usually caused slight venous dilatation. Histamine (0.5 μg/kg) caused arterial constriction; this effect was blocked by mepyramine (0.5 mg/kg).

Most experiments were made with the cannula tied into the right common carotid artery; in these conditions much of the blood supply to the ear came from the left side of the body through anastomotic channels. This disturbance of the circulation was not responsible for the changes observed in the ears of infected animals since injection of contrast medium by way of the mandibular or subclavian artery gave the same radiological appearances. It was almost impossible to obtain satisfactory angiograms of the ear by injection into the aortic arch. Owing to arterial constriction in the ears of infected rabbits it was difficult to obtain good angiograms of the veins. Accordingly, these vessels were studied in the cremaster muscles which are thin enough to enable whole preparations to be examined.

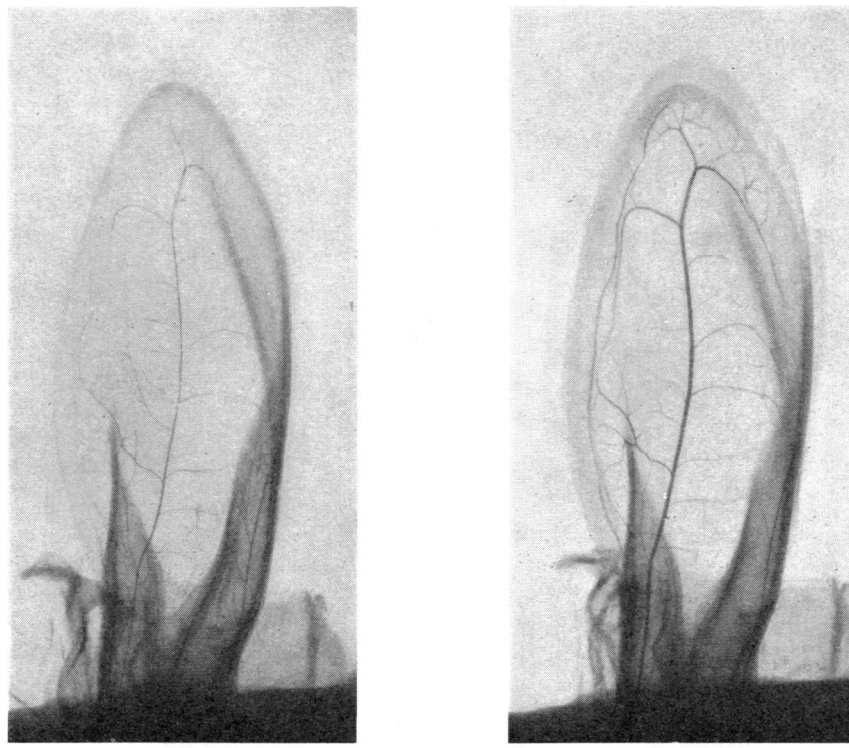


Fig. 2. Angiograms of the ear of a rabbit infected with *T.(T.) brucei* (a) before and (b) 1 min after intra-arterial injection of phenoxybenzamine (1 mg/kg). (×0.75.)

Blood vessels in the cremaster muscles

Cremaster muscles of normal rabbits injected with India ink showed a regular vascular pattern and very few carbon particles could be found on microscopical examination. The venous system in muscles from infected animals was dilated and tortuous and there were patches of vascularization at the sites of granulomatous adhesions. The vessels appeared dark to the naked eye and under the microscope were lined with carbon (Fig. 3). Almost all the carbon particles were enclosed in phagocytes that lined the venules and capillaries (Fig. 4); the arterioles were almost free from phagocytes. The appearance was similar to that of a rat cremaster muscle in which vascular injury had been produced several months previously by a local injection of histamine, as described by Majno (1964). Histological sections of cremaster muscle and other voluntary muscles from infected rabbits showed patches of granulomatous tissue, composed predominantly of mononuclear cells and rich in capillaries, surrounding dilated veins (Fig. 5). Similar changes were seen in histological sections of the ears of infected rabbits.

DISCUSSION

The results of the angiographic and histological studies on the ears and cremaster muscles of infected rabbits show that the lesion associated with *T.(T.) brucei* trypanoso-

miasis is a chronic angiitis. The venous drainage of muscles and viscera is congested, the tissues are oedematous, the walls of the blood vessels are fragile and surrounded with granulomatous tissue, characteristic of chronic inflammation. Venules and capillaries are lined with phagocytes, suggesting long-standing vascular damage. In the ear and elsewhere, arterial contriction, mediated through the autonomic nervous system, produces areas of anoxia, leading to tissue necrosis.

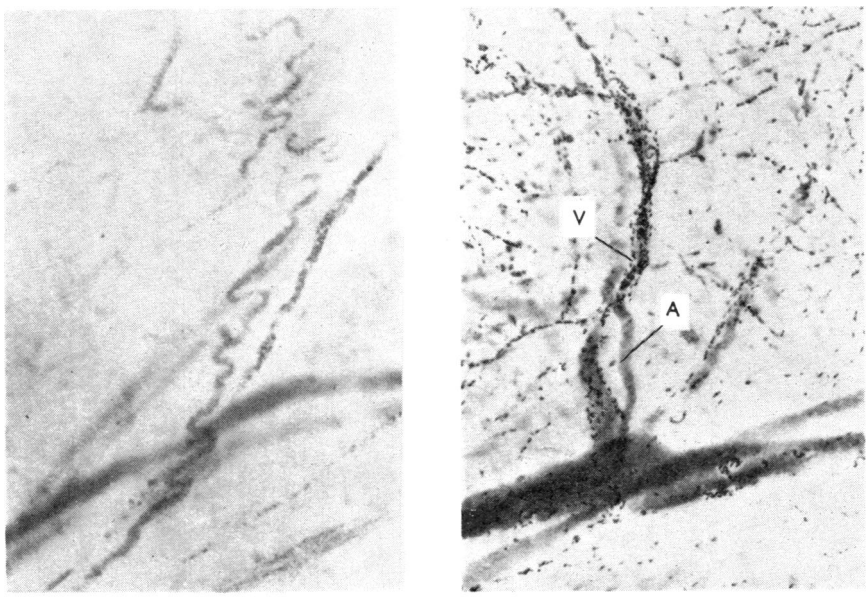


Fig. 3. Cremaster muscles of (a) a normal rabbit and (b) a rabbit infected with *T.*(*T.*) *brucei* after intravenous injection of India ink. In the normal rabbit the vessels contain erythrocytes but no carbon particles. In the infected rabbit the veins, venules (V) and parts of the capillaries are "tattooed." The arterioles (A) are not marked with carbon particles. (×40.)

A similar condition was described by Fiennes (1946) in cattle with relapsing *T. congolense* infections. Thrombosis occurred in many of the smaller blood vessels, the vascular endothelium was deficient and swollen, and macrophages were strikingly numerous in all connective tissues, especially the adventitia of the blood vessels.

Skirrow, Chongsulpajaisiddhi & Maegraith (1964) showed that portal vasoconstriction occurs in monkeys with acute *Plasmodium knowlesi* infections; the vessels relax when hexamethonium or phenoxybenzamine is given.

In acute inflammation the immediate response to injury is probably brought about by released histamine (Wilhelm, 1962) which causes increased vascular permeability, especially of the venules. The sustained increase of permeability which follows may be associated with released kinin, although this is difficult to prove (Willoughby, 1967).

The stimulus that causes cellular injury and initiates the pathology of most infections is not yet known. There may be a clue in the observation that serum from monkeys

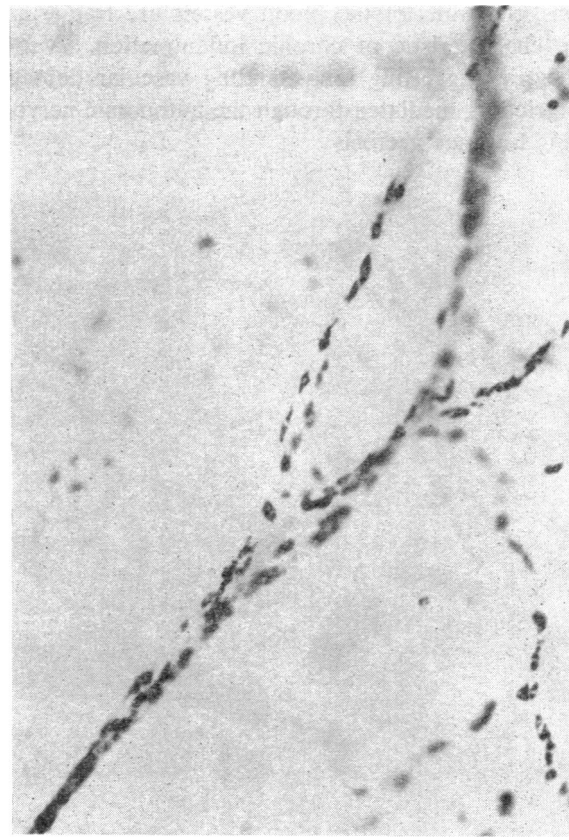


Fig. 4. Venules in the cremaster muscle shown in Fig. 3b. The carbon particles are contained in phagocytes that line the vessels. (×*ca.* 132.)

heavily infected with *P. knowlesi* contains a fraction which inhibits the respiration and oxidative phosphorylation of liver mitochondria (Maegraith, 1966). There is, however, no reliable evidence that trypanosomes contain toxins and in chronic *T.(T.) brucei* infections in rabbits the number of circulating parasites is small. Trypanosomes do contain antigens and it is likely that immune responses are associated with the pathology of chronic infections.

Fiennes (1950) suggested that in relapsing *T. congolense* infection in cattle, the massive destruction of trypanosomes at each crisis might sensitize the animal to dead trypanosome protein and, at each crisis except the first, anaphylactic shock might be induced. It was also suggested that trypanosome protein could act as an allergen, sensitizing vascular endothelial cells and causing them, on activation, to disintegrate.

In rabbits infected with *T.(T.) brucei* 427, few organisms appear in the peripheral blood and there are no apparent crises, but it has been shown (Gray, 1962) that as the infection progresses, a succession of antigenic variants appears. About 10 days after

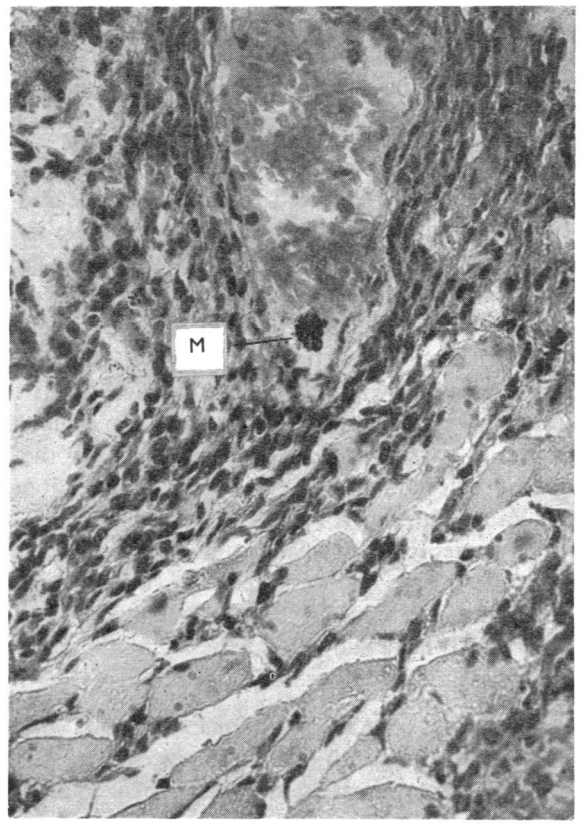


Fig. 5. Section of the cremaster muscle of a rabbit infected with *T.(T.) brucei*. (Haematoxylin and eosin ; ×150.) The muscle fibres are separated by granuloma cells. A macrophage (M), loaded with carbon particles, is attached to the endothelium of a venule.

inoculation, antibodies to the strain inoculated appear in the serum of the host. Thereafter, successive antibody peaks can be detected in the serum, each coinciding with the emergence of a new antigenic variant of the parent strain. Each rise in antibody titre corresponds with the disappearance of the homologous variant and is followed by the appearance of a new one. The production of antigenically variable trypanosomes depends on the ability of the host to produce antibody and there seems to be no end to the number of variants and the consequent antibodies that can be produced (Weitz, 1962). A similar series of variants appears in cattle infected with *T.(T) brucei* (Cunningham, Van Hoeve & Grainge, 1965). Animals with infections of this kind undergo a series of immunological insults not unlike those in Arthus's original demonstration of *accidents tardifs de la séro-anaphylaxie*. He found that a sensitized rabbit which survived the anaphylactic reaction after the intravenous injection of horse serum seemed normal after about 15 min. But a few days later the rabbit became thin, the fur dry, ruffled and loose and the skin scaly. The animal was inactive, the eye dull and the ears were drooping and pale. An incision made near the border of the ear gave only a small drop of blood. The leucocytes

were increased in number, the erythrocytes diminished and the haemoglobin decreased. Often necrotic patches appeared in the skin, particularly in the pelvic area. The rabbit finally died in a state of marasmus after a few weeks without having shown any further acute reactions (Arthus, 1903). The syndrome is in many respects similar to the course of chronic trypanosomiasis in rabbits and cattle, and we are grateful to Professor R. R. A. Coombs for drawing our attention to this.

Brocklehurst & Lahiri (1962, 1963) showed that kinin is released during anaphylaxis. The appearance of trypanosomal antibody in rabbits and cattle is also associated with the appearance of free kinin (Boreham, 1968) and at the same time plasma kininogen reserves are depleted. It is likely that there is a massive release of kinin and a rapid rate of turnover. Released kinin would be expected to have some effect on permeability and tone of blood vessels before destruction by kininase. Other substances, including histamine and SRS-A are released by antigen-antibody reactions (Brocklehurst, 1960) and their effects on the circulatory system would contribute to the difficulties of pharmacological analyses. Reflex arterial constriction may be reinforced by the presence of released histamine and perhaps by catecholamines released from the adrenal medulla by kinin (Staszewska-Barczak & Vane, 1967). Prolonged arterial constriction would produce areas of tissue anoxia in which proteolytic enzymes would be released and the local pH might fall sufficiently to favour the release and persistence of further quantities of kinin (Edery & Lewis, 1962; Greenbaum & Yamafuji, 1966; Greenbaum & Kim, 1967). Hyashi, Tokuda, Ono & Takaba (1963) have shown that the Arthus reaction activates a protease that may play a part in the inflammatory reaction.

Maegraith (1966) suggests that in acute infections such as *P. knowlesi* in the monkey, vascular changes take place that result in interference with tissue function; anoxia increases the permeability of small vessels and leads to a chain reaction that destroys the host.

It seems likely that the progressive vascular changes that accompany chronic *T.(T.) brucei* infection in the rabbit are initiated and maintained by allergic reactions.

SUMMARY

1. Angiographic studies of the ears of rabbits infected with *Trypanosoma (Trypanozoon) brucei* show that the arteries are narrowed. The vessels widen to almost normal diameter after administration of hexamethonium, phenoxybenzamine or atropine which shows that the infection causes arterial constriction through the mediation of autonomic nerves.

2. The venous system of the cremaster muscles of infected rabbits is congested and shows evidence of long-standing vascular damage.

3. It is suggested that vascular damage in chronic rabbit trypanosomiasis is caused by pharmacologically active substances released from the tissues as a result of allergic reactions.

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