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Miscellanea

Evidence of Intrauterine Transmission of a Trypanosome in Cattle[1]

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In our virology laboratory we routinely obtain fetal tissues of cattle from a local slaughter house for the preparation of tissue cultures. The tissues are removed aseptically from the fetus and brought back to the laboratory in clean sterile screw-capped jars. Standard techniques for isolating, washing and trypsinization of the tissues for cell cultures are followed. The medium used is Eagle's Minimum Essential Culture medium with 10% fetal calf serum. The pH of the medium was adjusted to 7.2.

In a recent isolation from the spleen of a calf (about 8 months gestation) we noticed trypanosomes in the spleen cell cultures 5 days after they were incubated at 37.5°C. The trypanosomes were dividing and all were trypomastigotes (Fig. 1). The trypanosome could be readily subpassaged but would not grow in the absence of a cell line. No trypanosomes were seen in the kidney cell cultures made from the same calf. Kidney cell cultures seemed healthy and there was no sign of bacterial or fungal contaminations. Trypanosomes from the spleen cell cultures grew well when inoculated into the kidney cell cultures and could be subpassaged without difficulty.

Smears of trypanosomes made from the cultures were air dried, fixed in 10% buffered formalin (Lehmann 1964) and later stained in Giemsa's stain. Sixty specimens were measured with the aid of a camera lucida (Table 1).

The anterior and posterior ends of the trypanosome are pointed (Fig. 1). The shape of the nucleus varies from round to oval and is located in the posterior region of the body. The large kinetoplast is usually round and its location between the posterior tip and the nucleus is variable. The undulating membrane is not extensive (Fig. 1). The length of the free flagellum is variable.

The trypanosome from the tissue culture closely resembles *T. americanum* as described by Crawley (1912). It differs in size from *T. theileri* and it does not produce epimastigotes in tissue culture medium incubated at 37.5°C as does *T. theileri*. Splitter & Soulsby (1967) reported that 30–40% of the forms of *T. theileri* in tissue culture medium were epimastigotes and similar results have been obtained in our laboratory.

Since the trypanosome grew well in the kidney cell culture, the absence of trypanosomes in the primary kidney cell culture was not due to any inhibitory substance that might have been present in the kidney cell cultures.

A plausible explanation for the absence of trypanosomes in the primary kidney cell culture might be that the trypanosomes occurred in larger numbers in the sinusoids of the spleen than in the capillaries of the kidney and despite the washings and trypsinization procedures employed in the preparation of tissue cultures there were still enough trypanosomes in the spleen preparations for the

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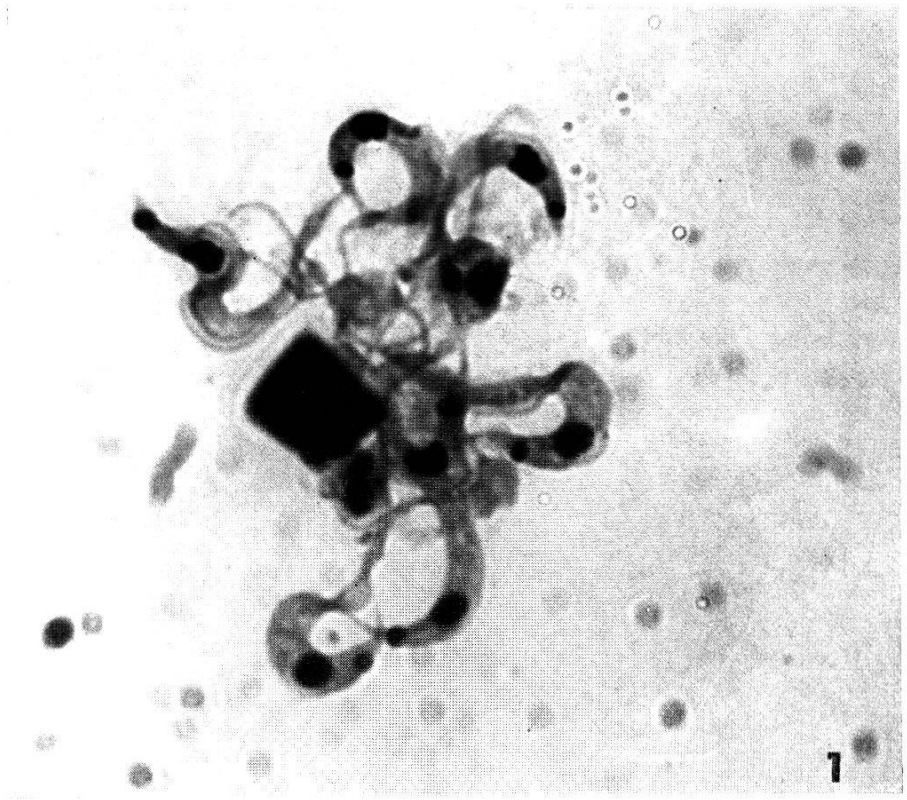

Fig. 1. Trypomastigotes of trypanosome growing in tissue culture medium, × 1,000.

organism to become established. Also, this trypanosome, like *T. brucei* or *Leishmania donovani* may have an intracellular stage in the cells of the spleen. Recently SOLTYS & WOO (1969) have shown this for *T. brucei* and that these amastigotes can be isolated and are infective (SOLTYS & WOO 1970) for mice.

Since the first report of SIVORI & LECLER (1902) on the intrauterine transmission of *T. equinum* in a pregnant guinea pig, there have been several reports of the intrauterine transmissions of pathogenic trypanosomes in man and in animals. These reports included females both naturally and experimentally infected during pregnancy. The literature has been adequately reviewed by DIRKMAN, MANTHEI & FRANK (1957).

Intrauterine transmission of *T. theileri* which is normally considered nonpathogenic, was first reported by DIRKMAN, MANTHEI & FRANK (1957). They found the trypanosome in the stomach contents of an aborted bovine fetus and attempts to culture the trypanosomes were unsuccessful. More recently LUNDHOLM, STORZ & MCKERCHER (1959) reported finding a trypanosome as a contaminant in a primary kidney cell culture of a bovine fetus. No morphological study of the

Table 1. Measurements of 60 trypomastigotes from culture

	PK	KN	AN	AP	BW	FF	PK/KN	PK/AP	AN/AP
Mean	3.7 μ	3.8 μ	11.6	19.1 μ	2.0 μ	11.6 μ	0.98	0.19	0.61
Range	2.1–6.4	2.8–5.7	8.5–13.5	16.3–24.8	1.4–2.8	7.8–14.2	0.50–2.28	0.09–0.47	0.52–0.68

trypanosome was made. They have indicated that the trypanosome was probably *T. theileri,* although they admitted a positive identification was not made. The fetus was not aborted but was removed from the uterus of the slaughtered cow.

Abbreviations in Table: PK, the distance from the posterior end to the kinetoplast; KN, the distance of the kinetoplast to the centre of the nucleus; AN, the distance from the centre of the nucleus to the anterior end; AP, the length of the body excluding the free flagellum; BW, the maximum width excluding the undulating membrane; FF, the length of the free flagellum.

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