

Correspondence

Failure to infect gnotobiotic, colostrum-deprived, and normal piglets with Treponema pallidum (Nichols)

TO THE EDITOR, *British Journal of Venereal Diseases*

SIR,—Since *Treponema pallidum* was incriminated as the causative agent of syphilis by Schaudinn and Hoffmann (1905), various attempts to grow the organism *in vitro* and in tissue culture have been unsuccessful (Willcox and Guthe, 1966). However, *T. pallidum* will grow in a variety of animals, including rabbits, monkeys, guinea-pigs, rats, mice, and hamsters (Turner and Hollander, 1957). Bertorelli was reported by Turner and Hollander (1957) as having observed a suspicious skin lesion in a pig 3 weeks after inoculation but another attempt to infect pigs was unsuccessful (Bessemans and de Wilde, 1935).

In this study, an attempt was made to grow *T. pallidum* in piglets of the Minnesota miniature variety. Piglets born normally, both colostrum-deprived and colostrum-fed, were studied as well as gnotobiotic (colostrum-deprived) piglets. Previous studies of these pigs by Kim, Bradley, and Watson (1966) have shown that gnotobiotic piglets obtained by aseptic hysterectomy 3 to 5 days before term lack immunoglobulins and that their tissues are free of 'background' antibody-forming cells. There should be, therefore, no immunological barrier to the introduction of *T. pallidum* in the form of cross-reacting serum antibodies. Since large numbers of *T. pallidum* are required for biochemical and immunological studies of this organism, it was hoped that a persistent bacteraemia would develop in these antibody-free piglets from which organisms could be obtained for study. Although fairly large numbers of *T. pallidum* can be grown in the rabbit testis, the treponemes are difficult to separate from rabbit tissue and are contaminated with rabbit protein.

All piglets were kept at a controlled temperature and were fed Mulsoy (Syntex laboratories, Palo Alto, California) by bottle every 6 hrs. They were inoculated with *T. pallidum* (Nichols) which was obtained from infected rabbit testes by elution in 5 per cent. bovine serum albumin (Reheis Chemical Co., Chicago, Ill.) in saline, diluted 1:2 with 20 per cent. glycerol in saline, and stored at -70°C . under nitrogen until required. The virulence of *T. pallidum* is maintained under these conditions (Turner and Hollander, 1957). When thawed the organisms were between 90 and 100 per cent. motile.

After infection, serum samples were taken from piglets at various intervals, diluted 1:2 in 20 per cent. glycerol in saline and stored at -70°C under nitrogen for later quantitation of *T. pallidum* by intradermal inoculation of rabbits (Turner and Hollander, 1957). For quantitative purposes the blood volume of a newborn piglet was assumed to be 50 ml. (Y. B. Kim, personal communication). Tissue samples from dead piglets were minced in a known volume of 5 per cent. bovine serum albumin in saline for 30 min. to allow elution of *T. pallidum*. The supernatant fluid was

diluted 1:2 in 20 per cent. glycerol in saline and stored as above.

To assay the serum and tissue elution samples for *T. pallidum*, 0.1 ml. samples were inoculated intradermally into the shaved skin of male Dutch Belt rabbits. Duplicates and triplicates were done in different rabbits, and all rabbits were kept at 16°C . for maximum lesion development. The time at which a skin lesion first becomes apparent is directly related to the number of virulent *T. pallidum* inoculated (Turner and Hollander, 1957). We have confirmed this observation and have used a graph based upon control tests with freshly isolated *T. pallidum* to determine the number of organisms in an unknown inoculum.

Four gnotobiotic piglets and one normal-birth colostrum-deprived piglet were kept at 32°C . and inoculated intraperitoneally with 10^8 *T. pallidum* 48 hrs after delivery. It was not possible to detect any *T. pallidum* in sera and tissues (spleen, lymph nodes, liver, kidney, testis, and peritoneal fluid) taken between 36 hrs and 14 days after infection. However, when five gnotobiotic piglets were kept at 26°C . and inoculated with 10^7 *T. pallidum* by various routes 9 hrs after delivery, it was possible to detect *T. pallidum* in sera 24 and 43 hrs after infection and in testes 43 hrs after infection (Table). By 43 hrs, all the piglets had died of non-infectious causes, including an uninoculated piglet.

The number of *T. pallidum* detected was considerably lower than in the inocula, suggesting that the *T. pallidum* were probably in the process of being cleared and would have been eliminated completely had the piglets been able to survive at 26°C . The percentage clearance of inoculated *T. pallidum* was over 99.9 per cent. (Table). Three normal piglets (10 days old and colostrum-fed) were kept at 16°C . and inoculated with 10^8 *T. pallidum* intraperitoneally, intravenously, and intratesticularly respectively. None of these pigs developed an infection, and sera taken 1, 2, 3, and 4 days post-inoculation were negative for *T. pallidum* by rabbit inoculation. A fourth normal piglet was inoculated with 2×10^8 *T. pallidum* intradermally (in duplicate) but no lesions appeared up to 16 days after inoculation. All four normal piglets kept at 16°C . appeared healthy and were unaffected by the lower temperature.

Sera from the various inoculated piglets, taken at intervals up to 6 wks, were all negative for reagenic antibody (RPR card test: Hynson, Westcott, and Dunning, Inc., Baltimore, Maryland) and *T. pallidum* immobilizing (TPI) antibodies. The absence of the latter indicates that the *T. pallidum* did not remain in the pig tissue long enough to stimulate a humoral immune response, while the absence of reagenic antibody suggests that no pig tissue reaction to the treponeme occurred (Turner, 1970). Although the piglets were monitored for only 6 wks after infection, rabbits are known to produce reagenic and

TABLE Isolation of *Treponema pallidum* from gnotobiotic, colostrum-deprived piglets, at 26°C., previously inoculated with 10⁷ *T. pallidum* at 9 hrs after hysterectomy

Group	Time after inoculation (hrs)	Number of <i>T. pallidum</i> positive skin lesions in rabbits from inoculation with pig sera or testes extract		Total viable <i>T. pallidum</i> in pig tissue and percentage loss of inoculated <i>T. pallidum</i>	
		Serum	Testes	Serum	Testes
(A) Intraperitoneal inoculation (3 piglets)	13	0/3			
	24	4/6		2.0 × 10 ⁸ 99.98%	
(B) Intratesticular inoculation (1 piglet)	24	0/3			
	43		3/3		3.2 × 10 ² 99.99%
(C) Intravenous inoculation (1 piglet)	43	3/3	0/3	8.0 × 10 ⁸ 99.92%	

immobilizing antibodies by this time (Turner and Hollander, 1957).

Temperature was a critical factor in the survival of the piglets. Newborn piglets are very susceptible to the cold, and the minimum temperature for their survival is about 29°C. Heat production by newborn piglets is low and does not reach maximum until 1 week of age. Furthermore, their body insulation is poor in the first few days of life (Whittow, 1971). Thus an ambient temperature of 26°C., at which some of the gnotobiotic piglets were kept, probably contributed to their death by 43 hrs after inoculation (52 hrs after hysterectomy). The *T. pallidum* were not cleared as completely from these piglets as from those kept at 32°C. This may have been due to a less effective phagocytosis in the dying piglets or more likely to an increased ability of the temperature-sensitive *T. pallidum* to survive at the lower pig temperature.

Temperature is a critical factor in the survival of *T. pallidum*. *T. pallidum* will survive at 35°C. but not 40°C. *in vitro* (Weber, 1953). *In vivo*, *T. pallidum* will remain viable in the lymph nodes of a rabbit (38°C.) but grows best in the shaved skin (35°C.) and testes (37°C.) (Turner and Hollander, 1957). These authors have concluded that the range in which *T. pallidum* multiplies *in vivo* is 30 to 38°C., with the optimum approximately 35 to 37°C.

At 32°C., piglet skin, blood, and rectal temperatures are 37.5, 39.6, and 39.7°C. and at 26°C., they are 34.2, 38.9 and 39.0°C. respectively (Ingram, 1964). It is apparent that at both 26° and 32°C. ambient temperature the piglet is too warm for the survival of *T. pallidum* with the possible exception of the skin. At 26°C. ambient temperature growth in the skin is theoretically possible since its lower temperature is close to the *T. pallidum* optimum. However, at 26°C., the newborn piglets did not survive longer than 52 hrs after hysterectomy. At 32°C. (gnotobiotic and colostrum-deprived) and at 16°C. (normal) the piglets did survive but skin lesions were never observed, even after intradermal inoculation.

Thus the failure of newborn piglets (gnotobiotic, colostrum-deprived, or normal) to support the multiplication of *T. pallidum* was strongly influenced by the high body temperature of the piglet. Since lesions failed to develop even in the skin, inhibitory nutritional or immunological factors were probably involved also.

Consequently, piglets are not satisfactory animals in which to attempt to grow *T. pallidum* in large numbers.

Summary

Normal piglets kept at 16°C. and gnotobiotic piglets kept at 32°C. rapidly destroyed *Treponema pallidum*, while gnotobiotic piglets kept at 26°C. showed slightly slower clearance. No infections were established. The high body temperature of the piglets has an important influence on the survival of *T. pallidum*.

Yours faithfully,

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References

- BESSEMAN, A., and DE WILDE, H. (1935) *C.R. Soc. biol. (Paris)*, **119**, 326
- INGRAM, D. L. (1964) *Res. vet. Sci.*, **5**, 348
- KIM, Y. B., BRADLEY, G. S., and WATSON, D. W. (1966) *J. Immunol.*, **97**, 52
- SCHAUDINN, F., and HOFFMANN, P. (1905) *Arb. GesundhAmte (Berl.)*, **22**, 527
- TURNER, T. B. (1970) 'Syphilis and the Treponematoses', in 'Infectious Agents and Host Reactions', ed. S. Mudd, chap. 16. Saunders, Philadelphia
- and HOLLANDER, D. H. (1957) 'Biology of the Treponematoses'. World Health Organization, Geneva
- WEBER M. M. (1953) Thesis, Johns Hopkins University, Baltimore
- WHITLOW, G. C. (1971) 'Ungulates' in 'Comparative Physiology of Thermoregulation, ed. G. C. Whittow, vol. 2, 'Mammals', chap. 3. Academic Press, New York
- WILCOX, R. R., and GUTHE, T. (1966) '*Treponema pallidum*: a Bibliographical Review of the Morphology, Culture, and Survival of *T. pallidum* and Associated Organisms'. *Bull. Wld Hlth Org.*, **35**, Suppl.