N. gonorrhoeae: pathogenicity of colonial type 5

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SUMMARY This paper records the results of the intravenous inoculation of chick embryos with type 5 gonococci and establishes that these strains are pathogenic.

Colonial variations of *Neisseria gonorrhoeae* have attracted considerable interest in recent times. In 1963 Kellogg *et al.* reported four different colonial types of *N. gonorrhoeae* and established a clear relationship between colonial morphology and virulence for human volunteers. It is now well accepted that Kellogg's colony types 1 and 2 are infective whereas types 3 and 4 are not. Jephcott and Reyn (1971) reported a fifth colonial type, which was believed to follow in sequence after type 4. Unfortunately, owing to the lack of any animal model or suitable human volunteers, the pathogenicity of colonial type 5 remained undetermined and, without any good evidence, came to be accepted as being akin to types 3 and 4.

During our study of the reversion of colonial types (Hafiz *et al.*, 1977) we observed that iron compounds were essential for reversion of colonial type 4 to 1 in ANM medium (Hafiz and McEntegart, 1976), whereas type 5 strains reverted to type 1 even in the absence of iron compounds. This led us to test the pathogenicity of type 5 strains by Finkelstein's (1964) technique of intravenous inoculation into 10-day-old chick embryos. This we believe has helped to establish the position of colonial type 5.

Material and methods

Colony types were identified by plating out on Difco GC medium plus 2% defined supplement consisting of 1·0 g glutamine, 1·0 g dextrose, 40·0 g ferric nitrate, 0·05 g, and cocarboxylase 0·02 mg in 100 ml distilled water (White and Kellogg, 1965).

Three colonial-type-5-forming strains of *N. gonorrhoeae*, including F62, were used in this study.

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The strains were confirmed by Gram's staining, oxidase reaction, carbohydrate fermentation, and fluorescent antibody technique. Different colonial types of each strain were selected and stored in liquid nitrogen. For experiments, gonococci of individual colony types 1, 4, and 5 were grown on the plating medium for 18 to 22 hours at 36°C in 10% carbon dioxide and increased humidity. The growth was checked for specificity of colonial types, harvested in phosphate-buffered saline (PBS), and diluted to contain 10⁶ organisms per ml using an EEL nephelometer, previously standardised by carrying out total viable counts according to the method of Miles *et al.* (1938).

For the test, 10-day-old embryonated eggs were used, and these were kept in a humidified incubator at 37°C. A rectangular window, approximately 3 × 5 mm, was cut over a prominent allantoic vein (while candling), and the shell flap was then lifted off with an 18 gauge needle. One hour after removal of the flap 0·1 ml of the appropriate suspension was inoculated into each egg with a 27 gauge needle; control eggs received 0·1 ml of PBS. After inoculation the eggs were returned to the incubator at 36°C and candled daily for three days. Suspected deaths of the embryos were confirmed by immediate examination. Specific endotoxin deaths showed typical pathological changes and usually occurred within 24 hours of inoculation.

Results

The Table shows the relative virulence of various colonial types of *N. gonorrhoeae* when inoculated into chick embryos. Identical results were obtained with different strains. It is evident from the results that the highest death rate occurs with type 1 (80%) whereas type 4 resulted in minimum (20%) mortality. Type 5 strains gave a 70% death rate, which is significantly higher than type 4.

Table Results of the intravenous inoculation of chick embryos with *N. gonorrhoeae* colonial types 1, 4, and 5

Colonial type	Total inoculated	Deaths	
		Total No.	Percentage
1	30	24/30	80.00
4	30	6/30	20.00
5	30	21/30	70.00
PBS (control)	30	0/35	0.00

Discussion

The intravenous inoculation of 10-day-old chick embryos with *N. gonorrhoeae* colonial types 1, 4, and 5 has helped to establish the relative virulence of colonial type 5. It is certainly more pathogenic than type 4 for the chick embryo, while slightly less pathogenic than type 1. It is interesting to note that this finding supports our earlier observations relating to the reversion of colonial types (Hafiz *et al.*, 1977) in that colonial type 5 reverted in ANM medium (Hafiz and McEntegart, 1976) whereas colonial type 4 required the addition of iron compounds before reversion occurred.

We suggest that *N. gonorrhoeae* colonial type 5 should be placed among the pathogenic colonial types, that is, just after types 1 and 2.

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