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Lethal challenge of gnotobiotic weanling rats with bacterial isolates from cases of sudden infant death syndrome (SIDS)

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SUMMARY An attempt was made to produce an animal model of sudden infant death syndrome (SIDS). The experimental animals (germ free weanling rats) were exposed to nasopharyngeal isolates from cases of SIDS to test the hypothesis that common bacteria may have an aetiological role in the disease. Negative results were obtained when the strains were tested in isolation, but certain combinations of organisms (specifically some *Staphylococcus aureus* and *Escherichia coli*) killed the animals rapidly (less than 18 hours) without prolonged terminal illness. Post mortem histological findings were consistent with those of SIDS. The lethal toxigenic potential of nasopharyngeal bacteria, which are regarded as harmless in adults, should be reconsidered in respect of the aetiology of SIDS.

Sudden infant death syndrome (SIDS) is the major cause of infant post-perinatal mortality in the developed world. Clinically, the diagnosis is made by exclusion, when a thorough post mortem examination has failed to identify any life threatening anatomical, histological, or microbial features. Epidemiologically, the syndrome has certain consistent features, including a peak winter incidence and an age incidence curve which rises from birth to a maximum at two to three months, then falls rapidly.

It has recently been suggested that SIDS is caused by common bacteria which normally colonise infants after birth.[1] SIDS might be a rare consequence of overwhelming infection or toxaemia occurring on first exposure to common, toxigenic bacteria as maternal IgG declines. The strong epidemiological link between SIDS and upper respiratory infections[2–4] might be accounted for by the overgrowth of respiratory tract bacteria which normally follows mucosal damage in a viral infection.[5,6]

This paper reports the results of preliminary studies designed to produce an animal model of SIDS based on this hypothesis. The aim of the work was to simulate the first encounter of the human infant with potentially lethal bacteria isolated from the nasopharynx of cases of SIDS, using experimental animals. We chose to use the germ free weanling rat.

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Material and methods

A pernasal swab was taken from cases of SIDS before necropsy, and as soon as the body was received (usually within a few hours of discovery). If delay was unavoidable the body was refrigerated. The swab was immediately plated on to a selection of standard media and incubated aerobically, anaerobically, or in 5% carbon dioxide, as appropriate. Plates were examined after 24 and 48 hours' incubation at 37°C. All colonial types were recorded, subcultured, identified and stored for further tests without any subjective assessment of their possible clinical importance.

The lethal potential of 28 nasopharyngeal isolates (representing various genera and species of bacteria) was tested in our experimental animal. In addition, certain species were tested in combination.

Test organisms were taken from an overnight culture on brain heart infusion blood agar, inoculated into 3–4 ml brain heart infusion broth in bijoux bottles, and incubated for five hours at 37°C; the number of organisms was approximated with the Miles and Misra technique.

For strains tested in isolation, 0·2 ml (equivalent to 10^9 colony forming units) of this culture was adminis-

tered directly without further treatment. In tests entailing combinations of organisms equal volumes of the component cultures were mixed immediately before administration, and 0·2 ml of the mixture was used.

All work was carried out with 21 day WAG/RIJ rats, reared under germ free conditions in plastic, thin film isolators. The animals were transferred aseptically to another isolator before the test to ensure first exposure to the test organism. Each group of four to six animals was inoculated and the experiments repeated in a similar number.

Initial experiments in which the test organisms were administered by nasal insufflation gave negative results. This might have been due to the inability of the organisms to colonise the healthy, uncompromised rat nasopharynx. The route of administration was therefore changed, and in all experiments reported here the test organisms were given by subcutaneous injection in the intrascapular region using sterile 1 ml syringes fitted with sterile 23 gauge needles. Control animals received an injection of 0·2 ml sterile brain heart infusion broth.

The animals were observed regularly throughout the experimental period for behavioural changes such as refusal to feed, drink or groom, or huddling alone; and for other physical signs such as rapid breathing, which might indicate illness. Any deaths were noted and any animals showing prolonged illness or distress were humanely killed. All survivors were killed by ether inhalation on day 5. Postmortem bacteriological and histological examinations were carried out on all animals including controls. Evidence of septicaemia was sought by culture of the spleen which was surface sterilised by brief immersion in 10% povidone iodine solution, neutralised in sterile 0·5% sodium thiosulphate, macerated in a little sterile brain heart infusion broth and cultured on brain heart infusion blood agar. The plates were examined after incubation at 37°C. The remaining broth was also incubated and subcultured at 24 hours if no growth was seen on the direct culture plate. Histological examination was carried out on formalin fixed tissue from the liver, kidney, pancreas, lung and heart. The tissues were embedded in paraffin wax and sections were stained by haematoxylin and eosin.

Table 1 *Tests on single strains*

Bacterium	*No of strains tested*	*Effect*		
		Lethal	*Local*	*None*
E coli	5	4	0	1
Citrobacter spp.	2	0	0	2
Klebsiella spp*	3	0	0	3
H influenzae	1	0	0	1
S aureus	7	0	5	2
Coagulase negative staphylococci	4	0	4	4
Streptococcus spp†	6	0	0	6

**Klebsiellia pneumoniae* (n = 2); *Klebsiellia oxytoca* (n = 1).
†*S bovis* (n = 1); *S pneumoniae* (n = 1), *S mitis* (n = 2), *S pyogene* (n = 1), *S faecalis* (n = 1).

Results

TESTS USING SINGLE STRAINS

Twenty eight nasopharyngeal isolates were tested. The only lethal organisms were four strains of *Escherichia coli*—the animals developed obvious illness on the second day after injection and deteriorated gradually. Those animals which died as a result of infection did so within 42–48 hours of challenge. The test organisms were recovered from the spleen after death. Some strains (five of seven) of *Staphylococcus aureus* produced local inflammation at the site of injection, with hair loss and abscess formation occurring over a period of a few days, but they produced no systemic effect (table 1).

TESTS ON COMBINATIONS OF STRAINS

Table 2 shows the composition of the mixtures tested initially, together with their effect on the weanling rat. Combinations of *E coli* and *S aureus* produced death within 18 hours of challenge without prolonged terminal illness. Simultaneous administration of *E coli* and streptococci had no such effect. Further studies on *E coli* and *S aureus* showed that this was not an invariable phenomenon but was restricted to certain combinations (table 3).

BACTERIOLOGY

The test organisms were recovered on direct culture from the spleen in all cases in which the animal died on test (or had shown signs of terminal systemic ill-

Table 2 *Composition of mixtures and their effect on animals: preliminary tests*

	S aureus			*Streptococcus*		
	1	*2*	*3*	*bovis*	*pneumoniae*	*pyogenes*
E coli	Sudden rapid death	Sudden rapid death	Sudden rapid death*	Death (42–48 hrs)	Death (42–48 hrs)	Death (42–48 hrs)

*Sudden onset of illness rapidly proving fatal (within 18 hours).

Table 3 *Combinations of E coli and S aureus*

	E coli strain		
S aureus	*1*	*2*	*3*
A	Sudden rapid death	Death	Sudden rapid death (80%)
B	Sudden rapid death	Death	Sudden rapid death (50%)
C	Sudden rapid death	Death	Death
D	Sudden rapid death		
E	Death		

ness before being humanely killed). In cases of death due to administration of mixtures of *E coli* and *S aureus, E coli* was invariably recovered in large numbers; staphylococci were not always found.

HISTOLOGY

The rats which died after prolonged septicaemic illness caused by a single strain showed a mild to moderate infiltration of inflammatory cells in the interstitium of the lung, the liver sinusoids, and in a diffuse distribution in heart muscle. The inflammatory infiltrate included neutrophils, lymphocytes, and histiocytes. There was Küpffer cell hyperplasia in the liver and some alveolar cell hyperplasia in the lung. In some sections bacterial colonies were visible. The rats which died rapidly after a short illness caused by combinations of bacteria showed a similar picture, although the inflammation was less intense. In addition, these animals showed focal ballooning necrosis of renal tubular cells.

The rats which survived bacterial exposure showed either no change or only very minor inflammatory changes in a similar distribution to the above. These changes could not be reliably distinguished from those in control rats which had not been exposed to bacteria.

Discussion

We have been partially successful in simulating SIDS in the germ free animal. While some deaths with single isolates were septicaemic with a short terminal illness, the deaths after inoculation of certain combinations were sudden with no terminal decline.

We found minimal histological evidence of respiratory inflammation compared with that of controls, a situation analogous with SIDS. Bacteria were, however, recovered after death, indicating that the animals had died with septicaemia rather than pure toxaemia.

LETHAL SYNERGY AND ITS CLINICAL RELEVANCE

Lethal synergy between micro-organisms in experimental models has been shown previously. Carlsson noted that *Candida albicans* substantially enhanced the establishment of murine infection with *Serratia marcescens* and *Streptococcus faecalis*[7] and increased mortality with toxic shock syndrome associated with *S aureus*.[8 9] In the latter case she suggested that the lethal mechanism might involve endotoxins which were either derived from *C albicans* or from the gut of the host (due to an increase stimulated by yeast in histamine production with concomitant leakage from gut to bloodstream, or inhibition of mechanisms clearing endotoxin from blood). This particular phenomenon of potentiation of endotoxin by toxic shock syndrome toxin (TSST–1) producing strains of *S aureus* has been described by Schlievert.[10] In our own study 40% of cases of SIDS carried *S aureus* in large numbers in the nasopharynx; 10 strains were toxigenic for either TSST–1 (three strains) or one of the enterotoxins. One of the TSST–1 producing strains had been included in the animal experiments and found to induce sudden death in combination with *E coli*.

The synergistic mixtures of organisms used in these experiments were derived from several cases; we did not succeed in inducing rapid death with mixtures of nasopharyngeal isolates from any one infant. Although there is a strong epidemiological link between respiratory infections and SIDS, if synergistic combinations of bacteria are important then only one component of the mixture need be nasopharyngeal. The others could be from the gastrointestinal tract: bacteria and bacterial products are continually absorbed into the blood stream thoughout life but are quickly cleared by the liver and other organs. Occult bacteraemia is a relatively common finding in febrile but otherwise well children attending paediatric "walk-in" clinics. Teele *et al* reported that 10·5% of children, all without apparent focus of infection, showed positive blood cultures, bacteraemia being most commonly seen in infants between 7 and 12 months old.[11] McCarthy *et al* reported that one of 11 cases of children with urinary infection had associated bacteraemia.[12] If a peak of toxaemia from the nasopharynx fortuitously coincided with a peak of toxaemia or bacteraemia from the gastrointestinal tract or elsewhere, then sudden death could occur even though either component alone was not lethal. Furthermore, upper respiratory tract viral infection might precipitate simultaneous bacterial overgrowth in the nasopharynx and endotoxaemia caused by impairment of hepatic Küpffer cell function.[13]

No animal is entirely suitable for use in experiments modelling human disease. In this study our choice of the gnotobiotic rat was governed by the necessity to mimic first exposure to bacteria (con-

ventionally reared animals will have wide experience of common bacteria and their toxins). Even gnotobiotic rats, however, are exposed to bacterial antigens in their food and this may be a limitation of the system. We are also aware that experimental results from animals reflect species specificity, both in susceptibility to invasion and to bacterial toxins. We chose to use weanling animals to minimise as far as possible their exposure to dietary antigens, not because of developmental comparability between the 21 day rat and the 3 month human infant.

In conclusion, we have shown that common bacteria present in the nasopharynx of cases of SIDS are lethal to an experimental animal on first exposure. This lethality is enhanced when combinations of bacteria are used, such that the sudden death of the animal is similar clinically and histologically to SIDS in human infants. The hypothesis that sudden death might require simultaneous infection by two synergistic bacteria is not incompatible with current clinical knowledge.

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