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MiniReview

Common bacterial toxins and physiological vulnerability to sudden infant death: the role of deleterious genetic mutations

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Abstract

The common bacterial toxin hypothesis of sudden infant death syndrome (SIDS) is consistent with the epidemiological features of the condition including the age distribution, seasonal incidence, association with prone sleeping and with exposure to tobacco smoke. The hypothesis is supported by experimental evidence but there are two barriers to its acceptance: the speed of onset does not fit with conventional concepts of an infective process; furthermore, the hypothesis appears to offer a single explanation for what is regarded as a multifactorial disease. Concepts from information theory are used to explore these objections. Complex physiological systems process information and need a high level of redundancy to minimise error. Models show that deleterious mutations in such a system will interact synergistically. Environmental perturbations are most likely to cause failure (sudden death) in systems with several mutations. Models also indicate that mutation rates will pose a limit to the size of the functioning genome and, therefore, increased complexity in evolution depends on using old genes in new combinations rather than the chance appearance of new genes. The idea that we share our genes with the rest of creation (same genes but different combinations) leads to the following conjecture: for every receptor controlling the flow of information across a cell membrane there will be a bacterially coded molecule that can switch it off or on. Based on this premise, bacterial toxaemia could cause sudden death, merely the time it takes for a molecule to associate with or dissociate from its receptor. Regardless of the number of physiological systems involved in SIDS, the age distribution will have a unimodal peak corresponding to the age range during which infant serum IgG reaches its nadir. In this way, the two barriers to the common bacterial toxin hypothesis can be overcome: one explanation but multiple bacteria and toxins acting with variable speed on multiple target systems.

Keywords: Common bacterial toxin hypothesis; Sudden infant death syndrome; Information theory; Redundancy; Deleterious mutations

1. Introduction

The age distribution of sudden infant death syndrome (SIDS) is the most consistent and characteristic feature of the condition [1]. The risk of sudden death rises rapidly to a peak at 2–3 months of age. The risk then falls so that death is uncommon after 6 months and rare after 12 months. This risk profile is approximately reciprocal to infant serum IgG levels [2]. Immunoglobulin G protects against extracellular infections with organisms such as bacteria and also serves to neutralise bacterial toxins. The common bacterial toxin hypothesis of SIDS is based on these observations [3,4]. A mathematical model closely predicts the age distribution but only if the microorganisms responsible are common. This implicates common bacteria of the normal microbial flora rather than less common pathogenic viruses.

The nasopharyngeal bacterial flora in SIDS, as assessed at autopsy, is disturbed compared with age, gender and season matched normal healthy infants [5]. There is increased carriage of staphylococci, streptococci and Enterobacteriaceae in infants who have died suddenly. This study was conducted in the 1980s when most infants slept prone. The combination of prone sleeping and clinical upper respiratory tract infection

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(URTI) in otherwise normal healthy infants causes a similar change in the nasopharyngeal flora [6]. Enterobacteriaceae, which are rarely found in normal infants without URTI, are found in the early morning in infants sleeping prone and who have URTI, but disappear later in the day. This is presumably due to secretions pooling in the upper airways after an overnight sleep but clearing during the daytime.

Staphylococci and Enterobacteriaceae injected into gnotobiotic weanling rats act in synergy to cause rapid death by septicaemia [7]. At autopsy, there are no diagnostic morphological features. Toxins prepared from *Staphylococcus aureus* and *Escherichia coli*, isolated from SIDS cases show lethal synergy in a chick embryo model [8]. In addition, minute doses of nicotine interact synergistically with bacterial toxins to cause death in this model [9].

Endotoxin is commonly raised in post-mortem tissues and, therefore, its presence in SIDS is difficult to assess [10]. Immunoglobulin G antibody to the conserved core of endotoxin is decreased in SIDS and this could implicate consumption by endotoxaemia prior to death or low levels of maternal anti-endotoxin antibody transferred to the infant before birth [11]. The curlin protein, a subunit of curly fimbriae, is a colonisation/adherence factor common to most Enterobacteriaceae. This protein is found in the serum of SIDS infants [12]. The most compelling evidence for a toxin role in SIDS, however, is the observation that in over 50% of SIDS cases the staphylococcal pyrogenic enterotoxins are present in brain and other tissues at autopsy [13]. These toxins are only produced when the temperature is between 37 and 40 °C; therefore, their presence post-mortem indicates an ante mortem event.

The common bacterial toxin hypothesis fits with the key epidemiological features of SIDS including the age distribution, the seasonal pattern, the association with prone sleeping and with exposure to tobacco smoke [4]. It is supported by experimental evidence as indicated above but there are two major barriers of its acceptance:

1. The mode of death in SIDS is sudden, and this does not fit with conventional concepts of the rate of progression of symptoms and clinical signs in infection. In the CESDI SUDI study [14] 78.6% of SIDS infants (250 of 318) were well (Cambridge Baby Check score 0–7) in the 24 h prior to death. These infants were put to sleep and found dead with a median interval of 4.2 h, 8% were found dead within 1 h and 15% within 2 h. For 54 infants who died during daytime hours, the median interval from being seen well to being found dead was 1 h 10 min (interquartile range 23 min to 1 h and 52 min) (Fleming, P., personal communication). In the few infants that have died on a monitor the physiological events have gone from normal to death in under 20 min [15].
2. The one theoretical proposition with which all research workers concur is that causation in SIDS is multifactorial. Yet the common bacterial hypothesis, based as it is on an explanation of the age distribution of the syndrome, appears to offer a single cause.

In this article I intend to explore these two barriers, approaching the quest from first principles using concepts from information theory [16].

2. Information theory

If a physicist had been asked, at the beginning of the 20th century, ''Of what is the universe composed?''. He is likely to have answered ''Energy''. The same question at the beginning of the 21st century is more likely to elicit the answer ''Information and Uncertainty''. Energy is a fundamental concept. It is difficult to define because it has no component parts, but it can be measured. The history of the universe from the big bang to the present epoch is understood in some detail based on the concept of energy. Information is also a fundamental concept. It too is difficult to define because it has no component parts, but it can be measured. Information is measured as the reduction of uncertainty on a log scale with base 2; thus one bit of information reduces uncertainty by one half.

The impetus to study information came from telecommunications engineers who wanted to measure that which they sent along telegraph wires and through the ether. Alan Turing, a Cambridge mathematician, was an important figure in the history of information. He was involved in breaking the enigma code in the Second World War and went on to develop the digital computer. A mathematical theory of information was published in 1948 by Shannon [17], another Cambridge mathematician. This gave engineers a way of measuring information, but it also specified the link between information and uncertainty. In 1953, Crick and Watson showed that DNA was the molecule of information and ushered in the molecular biological revolution.

Alan Turing was interested in the mind. He thought the mind was a machine and he planned to produce a machine that could think, i.e., the digital computer. It is probably for this reason that the first biologists to be interested in information theory were experimental psychologists. They reasoned that the brain was an information processing system as was the digital computer; so the general principles that apply to all information processing systems would apply to both, and the study of one would inform the other. The general principles are as follows [16]:

1. All information systems have a finite capacity.
2. Information is processed in a background of noise and errors will occur.

3. Systems deteriorate with time according to the laws of entropy and the error rate will rise with time.
4. All complex information processing systems need a high level of redundancy in order to reduce errors to a minimum.

The brain processes information but so does the immune system. The vast array of epitopes on proteins of the microbial flora must be classified into self and not-self. This is a decision system operating in an uncertain world and the above principles will apply. We can go further. All complex physiological systems involve the flow of information along nerves and across synapses. The cells that constitute the system must communicate and act in concert or the system will fail; thus all complex physiological systems are information processing systems and they will conform to the general principles listed above.

3. Deleterious mutations

There are approximately 30 000 genes (haploid set) in the human genome [18]. Each individual carries a number of deleterious mutations (let the mean number per adult = Y). In each generation new deleterious mutations arise in spermatogenesis, and to a less extent in oogenesis, and are passed on to human progeny (let the mean number of new deleterious mutations per zygote = X).

An estimate of the value of Y can be obtained from the frequency of recessive disease in the offspring of cousin marriages using the following equation [19]:

Probability of recessive disease

$$= 1 - (1 - [Y^6/2^6(Y+X)^6])^{2Y}.$$

The frequency of recessive disease in the offspring of a sibling union is

Probability of recessive disease

$$= 1 - (1 - [Y^4/2^4(Y+X)^4])^{2Y}.$$

The frequency of a specific rare recessive disease in cousin unions divided by its frequency in the general population is given by the following function:

$$(P)Y^5/16(Y+X)^6,$$

P = the number of genes in the human diploid set.

The rate at which base changes occur in non-coding sections of the genome can be extrapolated to coding sections to estimate the rate at which new mutations arise. This gives an estimate of X as greater than one [20]. An estimate of X and Y has also been made using the third function listed above [19]. This estimate placed X between 1 and 2.6 and Y between 1 and 32 but the value of P used was 120 000 which is now thought to be too high. If $P = 60000$ and X is >1, then Y is between 3 and 11.

If $X = 1$ and $Y = 10$, then using the first two functions listed above, 16.2% of the offspring of cousin unions and 58.2% of the offspring of sibling unions would have some form of recessive disease. These figures are too high [21]. One study showed that first cousin progeny suffered 4.4% more pre-reproductive deaths than the offspring of non-consanguineous unions [22]. A second reported 10% more major malformations in the offspring of first cousin unions [23]. If $X = 1$ and the frequency of recessive disease in the offspring of cousin unions is between 4% and 10% then Y will be between 4 and 8.

There must be some form of selection against deleterious mutations in the heterozygote as well as the homozygote, otherwise the value of Y would increase to a level at which consanguineous unions were sterile. If selection only operates against the homozygote, any single mutant gene will reach a frequency of $1/(P)^{0.5}$ if $X = 1$, this would result in values of $Y > 240$ and progeny free of recessive disease would not occur in first cousin marriages.

4. Redundancy

Complex physiological systems are specified by genes. They need a high level of redundancy to function adequately; but if a gene specifies a component of a highly redundant system, then mutation of that gene will have no measurable or discernible effect on function. How can selection operate against the mutation? How can a progressive build up of deleterious mutations be prevented? How can redundancy be preserved? These questions have been addressed in a recent paper using mathematical models of redundant systems specified by genes [24].

Consider a unicellular, asexual, haploid organism (organism A) which fights a potentially lethal infection by a parasite using a highly redundant system. The redundant system has m components, but i of them are inactivated by deleterious mutations. Organism A is attacked by q parasites and the probability that one parasite can evade any one active component is p. If a parasite evades all $(m - i)$ active components, organism A is killed. Each component is specified by a set of genes, and deletion of one gene by mutation deletes the component.

The probability that organism A survives the parasitic attack is $(1 - p^{m-i})^q$. In Fig. 1, the probability of survival is plotted against the number of deleted components, assuming $m = 7$ and $p = 0.1$. The survival curves are for $q = 1000$ and 100 000. The survival curve is synergistic. When $q = 1000$ the deletion of one or two components has no measurable effect, a third deletion has a small effect but a fourth has a marked effect. In this example, there would be selection against

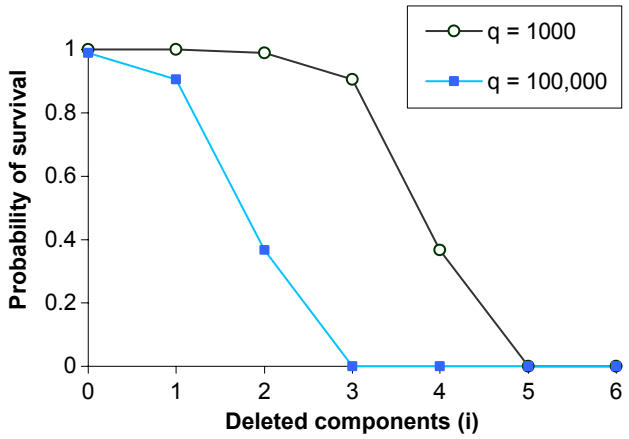

Fig. 1. The probability of survival of a complex, highly redundant system under attack by q parasites: deleted components interact synergistically.

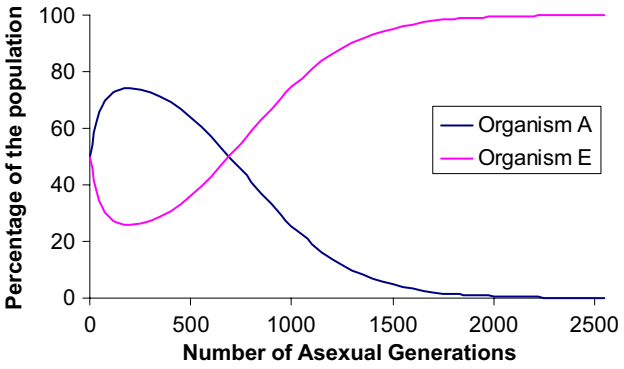

Fig. 2. Organism A (asexual) and organism E (sexual) are grown together in a finite environment.

organisms with four deletions but not one, two or three. When the environment is more threatening ($q = 100\,000$) the degree of redundancy is less and even one deletion has a small effect.

A computer model has been developed to follow the fate of organism A through a large number of generations. Individual organisms are attacked by q parasites. If they survive the attack, they then divide by mitosis. The mutation rate at mitosis is b per organism per cell division. The cycle then repeats for the daughter cells. If $b = 0.1$ or 0.01, the asexual organism loses the highest levels of redundancy after several thousand generations, and even in very large populations, there are no remaining organisms without at least one deleted component. If this population is then subject to a 10-fold or 100-fold increase in the value of q, all the organisms will be destroyed and the population will become extinct. In an asexual population there is a gradual build up of deleterious mutations and loss of the highest levels of redundancy. The organism can reach a stable equilibrium in a stable environment and survive long term; but if the environmental conditions worsen, the organism risks extinction. If $b = 0.001$, as seen in bacteria, then 50% of the organisms retain the highest levels of redundancy and can survive long term.

Organism E is identical to organism A apart from a sexual phase. After a number of mitotic divisions, haploid cells fuse to form a diploid cell which then divides by meiosis to form haploid progeny. Organism E reaches a stable equilibrium in which there are always a few cells with no deletions; thus the highest levels of redundancy are retained in a proportion of the population and the organism can survive long term. If organisms A and E are grown together in a finite environment with finite resources, then organism E will outgrow and completely replace organism A. This is illustrated in Fig. 2. The advantage of sexual reproduction is that deleterious mutations are distributed at random to the next generation, those with less than average survive and grow, those with more than average are more likely to succumb and die. In sexual reproduction, some of the progeny will have fewer mutations than their parents and, therefore, the progressive build up of deleterious mutations is reversed.

Mechanisms of DNA repair are conserved in evolution and the error rate per base pair per cell generation is similar in unicellular and multicellular organisms [25]. As genomes get larger, the mutation rate per cell per division increases. If increasing complexity depends on increasing genome size, this creates a major problem for asexual organisms in that an increased mutation rate leads to a loss of redundancy and, therefore, a loss of complexity. This limits the extent to which complexity can be increased by increasing the size of the genome; however, if old genes are used in new combinations to produce new capabilities, there is no limit to the increase in complexity. In this respect, it is useful to note that bacteria have 5000 genes, yeast have 6000, flies have 13 000, worms have 18 000, plants have 26 000, and mice and men have 30 000 [18]. Increasing complexity in evolution is not proportional to genome size; it must depend on using old genes in new ways.

In humans, sexual reproduction distributes deleterious mutations at random to gametes which then fuse at random to form zygotes. The result is close to a Poisson distribution of deleterious mutations in zygotes with a mean of $X + Y$ (Fig. 3). As in the unicellular haploid organisms modelled above, the zygotes with the most deleterious mutations are the least fit and the ones most likely to die. The distribution of mutations in survivors is shifted to the left and the mean is Y. The population is in balance: adults have a mean of Y, zygotes have a mean of $Y + X$; selection operates at the zygote stage and survivors have a mean of Y.

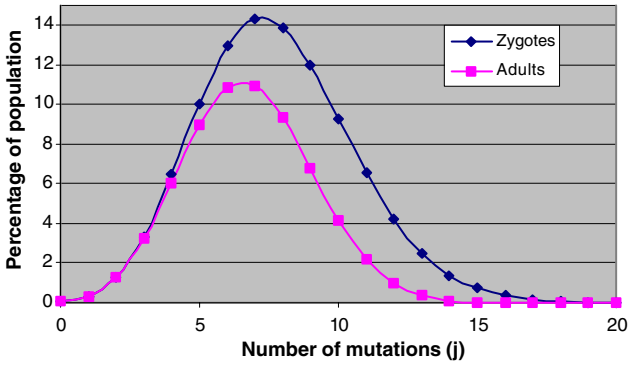

Fig. 3. The distribution of deleterious mutations in zygotes and adults.

5. System Z

Consider a complex physiological system specified by a large number of genes. The mean number of deleterious mutations in the system in the general population could be close to 0.5, if 6000 genes specify the system, or perhaps 0.1 if 1200 genes specify the system. If the mean is 0.5, 60% of the population will have no mutations, 30% will have one mutation, 8% will have two and 2% will have three or more. If environmentally determined perturbations of system Z can cause sudden death in infants, then death is most likely to occur in those with three or more deleterious mutations. Those with two mutations will be at lower risk and those with one or none at much lower risk. In a complex redundant system, deleterious mutations will confer physiological vulnerability to environmentally determined disease.

6. Bacterial toxins and the microbial flora

The 30 000 genes in the human haploid set specify up to 500 000 proteins [26]. By comparison, *E. coli* has 5000 genes specifying 5000 proteins. The difference in complexity is related more closely to the proteome size than the genome size.

The microbial flora of the respiratory and gastro-intestinal tracts consists of very many different species, each with up to 5000 genes. It appears likely that the total number of different genes carried by the microbial flora will exceed 30 000. The model of genetic redundancy described above [24] indicated that increasing complexity in evolution was due to using old genes in new combinations rather than increasing the genome size with new genes. The implication is that we share our genes with the rest of creation, and species specificity depends on the way the genes are combined.

Receptors in human cell membranes are composed of a number of protein subunits specified by several genes. In addition, there are agonist and antagonist molecules for each receptor also specified by genes.

If the above analysis is correct, it leads to an interesting conjecture. All our genes, or at least the majority, will have close relatives that are carried by bacteria of our normal flora; therefore, for every receptor that controls the flow of information across a cell membrane, there will be a bacterial coded molecule that can switch it on or switch it off.

7. Discussion

In this article I have argued that physiological vulnerability will be due in part to deleterious mutations in the genome. All physiological systems are fallible and there is a finite risk of failure; redundancy reduces the risk but deleterious mutations reduce redundancy; thus whatever the environmental events are that can cause sudden death, they are more likely to succeed in infants with genetic deletions in the particular system.

If we share our genes with the rest of creation, and the redundancy model indicates that this is likely, then bacterial products will be capable of interfering with flow of information across cell membranes. Bacterial toxaemia, due to absorption from mucosal surfaces or secondary to bacteraemia, will, therefore, perturb physiological systems; and this will be maximal when protective IgG antibody is at its lowest levels. The speed of on-set and the speed of off-set of the perturbation will depend purely on the time it takes for a molecule to combine with or dissociate from a receptor. The sudden onset of symptoms in SIDS and the rapidity of death is not inconsistent with a toxin-induced mechanism.

Even if SIDS is multifactorial with many different physiological systems involved and many different bacterial toxins in different combinations, the age distribution will still be uni-modal with a sharp peak when IgG is at its nadir; thus the multifactorial nature of SIDS is also not inconsistent with the common bacterial toxin hypothesis.

Of the mutations that occur during spermatogenesis and oogenesis, most are deleterious but a few are neutral. There is, however, no selection against neutral mutations; they accumulate in the genome through successive generations and constitute the main form of genetic diversity between individuals [27,28]. Susceptibility to infection is influenced by neutral changes in the genome in that individuals can be at increased risk from certain microbes but at decreased risk from others; overall the effects on survival are neutral. If common bacterial toxins have a role in the pathogenesis of SIDS, it could well be that neutral mutations also play a part. The argument that deleterious mutations confer susceptibility to SIDS does not in any way lessen the potential importance of neutral changes.

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