REVIEW

PATERNAL CONTRIBUTION TO EMBRYONIC DEATH*

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Summary. This essay advances the theory that an unsuspectedly large part of embryonic death is attributable to genetic causes. The genetic factors involved are not necessarily inherited by the parents, indeed the majority probably arise *de novo* in each parent generation and some are likely to arise in the definitive gametes. The theory seeks to account for the nature of unexplained fertility differences between males, and of idiogenic infertility, and of some of the decline in fertility that is associated with age. It suggests that a considerable part of embryonic death is unavoidable and should be regarded as a normal way of eliminating unfit genotypes in each generation.

INTRODUCTION

Most accounts of embryonic death appear to assume that blame lies entirely with the mother. This belief is so widespread that it has led, I believe, to an unbalanced and unsound approach to the problem of embryonic death and of its significance in reproduction. This paper presents a different point of view with the hope of provoking useful discussion and investigation. It suggests that a considerable part of embryonic death is attributable to the male, or to the mating system, and further that much of this loss is unavoidable and should not be regarded as pathological in any way. It should be said at once that most of what follows about the male contribution to embryonic death, could, with appropriate modification, also be said of the female. The emphasis placed here on the male reflects the author's personal approach to the problem and also serves the useful purpose of illustrating the extent to which the causes of embryonic death may be dissociated from the environment that is provided for the embryo by the female reproductive tract.

FERTILITY IN THE BULL

My interest in the possible influence of the male on embryonic death stems from an investigation of the fertility of bull semen carried out a few years ago (Bishop, Campbell, Hancock & Walton, 1954; Bishop & Hancock, 1955;

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Bishop, 1955a, b). In this investigation, a number of morphological and physiological characteristics of bull semen were examined and the fertility levels of the semen samples investigated were measured by artificial insemination trials. The accumulated data were then examined to determine the relationships between the measurements of the test characteristics and the measurements of fertility.

From this study, four points emerge which are relevant to the present thesis and provide the original background for the argument. These are:

(1) Although all the bulls examined had been specially selected for artificial insemination service and were of satisfactory fertility for this purpose, there were, nonetheless, significant differences in fertility between them.

(2) Some of the observed differences in semen characteristics were significantly related to the observed differences in fertility (in some instances $P < 0.001$), but in no case was this relationship a close one (i.e. the correlation coefficients were low). At best, only about 23% of the variation in fertility could be accounted for in terms of variation in the test characteristics, and 77% remained unexplained.

(3) There were significant differences in fertility between breeds of bull, but these differences were not related to the semen characteristics examined.

(4) There was evidence of a significant decrease in fertility with age of bull, but the only semen characteristic to which this was related was volume of ejaculate. Older bulls tended to produce ejaculates of larger volume.

The fertility of a bull, or of a semen sample, will be defined for the moment as its ability to procure successful conceptions. In cattle, which normally bear only one young at a time, the success of an insemination can be regarded as an all-or-nothing event and the definition is easy to apply. In the investigation referred to, fertility was measured as the conception ratio at 3 months. This is the proportion of cows, expressed as a percentage of the total inseminated, that are assumed to be pregnant (from records of re-inseminations) 3 months after insemination. The conception ratios at 3 months of bulls in artificial insemination service in this country vary from about 55 to 75%. Bulls of lower fertility are quickly eliminated from the studs and bulls of higher fertility do not exist. The frequency distribution curve of the conception ratios of these bulls is markedly skew, with a mode at about 70% (*see* Walton, 1958). The conception ratio of a bull must of course be ultimately limited by infertility factors in the cows inseminated and the late Dr Arthur Walton interprets the skew distribution of bull conception ratios as evidence that the fertility of any sufficiently large population of cows is about 75%. Walton (1958) calls this the "idiogenic fertility level" which he defines as "that level of fertility which is innate and depends on the total endowment which the individual receives at birth, whether genetical, embryological, physiological or environmental." Walton goes on to assume that the idiogenic fertility level of the average male is well above that of the average female and finds in this an explanation of the consistent failure of investigators to find satisfactory relationships between the semen characteristics and conception ratios of bulls that are at least moderately fertile. (There is usually little difficulty in detecting bulls that are sterile or of

low fertility by their semen characteristics.) The implication of Walton's hypothesis is that bulls with a conception ratio of 75% would have a conception ratio of 100% were it not for limitations imposed by 25% of the cows inseminated. It is known, however, that most of the cows that comprise this 25% present no detectable abnormality or pathological lesion and are not permanently sterile. The majority, in fact, readily conceive when re-inseminated and the conception ratios for second and third inseminations are not very much lower than those for first inseminations (Robinson, 1957; Ogden, 1959; Boyd & Reed, 1961). Furthermore, Walton's hypothesis appears to offer no explanation for the significant differences in conception ratio that are observed between bulls in artificial insemination service.

There is evidence that a considerable part of the reproductive failure of the 25% of cows that fail to conceive at first insemination is associated with embryonic death. Estimates of the incidence of embryonic death in cattle vary from 15 to 60% according to the source of the material examined, and embryonic death of a similar magnitude appears to be a feature of all other species of mammal, including man, that have been sufficiently investigated (Brambell, 1948; Laing, 1952, 1957; Casida, 1953, 1956; Roberts, 1956; Robinson, 1957; Hanly, 1961). The causes of embryonic death are doubtless several, but in the main they have been sought in deficiencies of the environment provided by the female reproductive tract. The suggestion made here, which is a development of an earlier thesis (Bishop, 1955b), is that a much larger part of embryonic death than is generally recognized is ascribable to genetic causes, and that a large part of the fertility differences between bulls in artificial insemination service is attributable to genetic factors carried by their spermatozoa which result in embryonic death but are not revealed in conventional tests of semen quality. Walton's idiogenic fertility level is thus seen as largely a consequence of embryonic death resulting from the reassortment of genetic factors at each fertilization—a theory which would seem to explain not only the upper limits of bull conception ratios, but also the observed differences in conception ratio between bulls. Unfortunately, the conception ratio at 3 months, useful though it is as an estimate of fertility, does not allow a distinction to be made between infertility caused by ovulation and fertilization failure and that caused by embryonic death during the first 3 months after insemination. The rest of this account seeks to make an *a priori* case in support of the hypothesis advanced above and to indicate the probable nature of the genetic factors involved.

There is, of course, an additional important way in which the male can influence embryonic death. This is by the venereal transmission of pathogenic organisms. In recent years, the importance of venereal disease, particularly of vibriosis, as a cause of prenatal death in cattle has become widely recognized (see Roberts, 1956). There is, however, no reason to believe that disease influenced the fertility of the bulls in our investigation. All were clinically examined and their breeding histories were well known. The disease aspect of the male influence on embryonic death will not, therefore, be further considered here, although it should be pointed out that some pathogens are readily transmissible by artificial insemination and do not necessarily affect semen characteristics in any obvious way.

GENETIC CAUSES OF EMBRYONIC DEATH

The biological significance of sexual reproduction lies mainly in the fact that each fertilization gives rise to a new combination of genetic material which can express itself as a new phenotype upon which natural selection can work. Without this there would be no speciation and no evolution in the sense in which we understand these terms today, and it is for this reason that sexual reproduction has been selected for and spread throughout the animal and plant kingdoms. Each fertilization may therefore be regarded as one of nature's genetic experiments and inevitably these experiments sometimes go wrong. The 'mistakes' that come to the attention of clinicians are those that manifest themselves fairly late in pregnancy, or after birth. Many such are known in both man and domestic animals and new techniques of chromosome analysis are constantly revealing previously unsuspected ones. The incidence of these genetically determined abnormalities is, however, low, and the most probable reason for this is that most of the 'mistakes' are eliminated by embryonic death. On *a priori* grounds one would expect this to be so, for obviously lethal factors that kill late in development are biologically very costly and natural selection would ensure that their incidence was kept as low as possible. This raises an important new concept; this is that some part of embryonic death, and probably a considerable part, should be regarded as a perfectly normal way of eliminating unfit genotypes, and if this is so, there is obviously no sense in trying to salvage this part of reproductive loss.

The principal genetic causes of embryonic death are lethal factors carried by the spermatozoon, or the egg, or both. Lethal factors are genes, or arrangements of genes, which result in death in the next generation before the reproductive phase is reached. They may be in the form of point mutations, deletions, replications, inversions or translocations. They vary in their degree of penetrance (i.e. frequency of manifestation) and in their degree of expressivity (i.e. strength of manifestation) and to an extent these are dependent upon the internal and external environments. Those which exert their effects only in certain circumstances are called conditional lethal factors. Those which are associated with the differential segments of the sex chromosomes are called sex-linked lethal factors. Those which are associated with the autosomes but cause death in one sex only are called sex-limited lethal factors. Lethal factors often exert their effect at a particular phase of development that is characteristic for each factor. Many are known to lead to the death of early embryos and almost certainly the majority operate during the early phases of development. (For further general information on lethal factors reference should be made to Hadorn's (1961) excellent monograph.)

Genetic factors that are transmitted by the male to the embryo may be further classified into three classes according to the time in the life cycle at which they are acquired. These classes are:

- (1) Factors that are inherited by the male from his parents.
- (2) Factors that arise in the spermatogenic tissue.
- (3) Factors that arise in spermatozoa after their release from the testis.

GENETIC FACTORS INHERITED BY THE MALE FROM HIS PARENTS

The lethal factors inherited by a male from his parents will obviously not include any dominant factors of full penetrance, nor any anti-male sex-limited factors of full penetrance, nor, in mammals, any sex-linked factors. They may well include dominant lethal factors of less than full penetrance, and factors (such as translocations) that become dominant lethals as a result of chromosome rearrangements at meiosis, and dominant factors of various kinds that are lethal in the homozygous state. They may also include a number of recessive lethal factors which often pass undetected and can be readily demonstrated only by special mating systems. Recessive factors are of special eugenic importance in animal breeding where one male can, by artificial insemination, sire many thousands of offspring and thereby distribute his genes throughout a large part of the total population. If the male is unwisely chosen, the economic losses can be enormous. A good example of this is provided by the Percheron stallion 'Superb', which in 1886 was exported from America to Japan. For two generations, the descendants of this stallion were satisfactory, but as soon as inbreeding started a large number of foals died within a few days of birth. The cause of death was soon established as atresia coli, a condition for which 'Superb' must have been heterozygous (Yamane, 1928). If, however, the lethal factor had operated during the embryonic stages of development, the only complaint against the descendants of 'Superb' would have been an unspecified form of infertility. It seems likely that many cases of unspecified infertility are of this nature.

The manner in which recessive lethal factors manifest themselves emphasizes an important inadequacy of our definition of fertility, for obviously in many instances in which genetic factors are concerned, fertility will be a characteristic of the mating system (i.e. which male is mated to which female) rather than of the individuals involved, and an awareness of this does appear to be fairly common among breeders of animals for it is often asserted that certain mating systems 'nick' whereas others do not. In addition to recessive lethal factors, the inherited genotype of the male may include other genetic factors of various kinds that lead to incompatibility and reproductive loss in certain mating systems. The resulting kinds of incompatibility may be divided into the following three classes:

- (1) Incompatibility between spermatozoa and dam.
- (2) Incompatibility between spermatozoon and egg.
- (3) Incompatibility between zygote and dam.

Incompatibility between spermatozoa and dam would be expected to lead to infertility caused by fertilization failure. Incompatibility of this type is well known in plants: it is not definitely known in animals but it could explain some known abnormal segregation ratios in certain mating systems (Bishop, 1960).

The recessive lethal gene is in a sense an example of incompatibility between the spermatozoon and the egg and many more complex situations are known in which the lethality of genetic factors are dependent upon the total genic environment (*see* Hadorn, 1961). Extreme instances of this kind are provided

by the failure of embryonic development that may follow interspecific fertilization. This occurs, for example, after cross fertilization between the rabbit and the hare (Adams, 1957) and between the rabbit and the cotton tail (Chang & McDonough, 1955). In each case the animals concerned are normally fertile within their normal mating systems, but the hybrid embryo is inviable.

The best known example of incompatibility between the genotype of the zygote and that of the dam is provided by Rhesus factor incompatibility associated with haemolytic disease of the newborn in human beings. This again is a condition that has become widely recognized on account of its manifestation at a late stage of development and it is probable that many more subtle instances of this kind await recognition. It is known, for example, that certain inter-blood-group matings within the ABO system in human beings are associated with losses of particular genotypes in the offspring (*see* Race & Sanger, 1958; Reed & Kelly, 1958; Matsunaga & Itoh, 1958; Chung & Morton, 1961). Similar situations appear to hold in respect of the J blood group factor in cattle (A. L. Ogden, personal communication, 1963) and of serum- β -globulin type in cattle (Ashton & Fallon, 1962), and Ogden believes that incompatible matings within systems of balanced polymorphism may account for a large proportion of reproductive loss.

The inherited load of lethal and incompatible factors will, of course, vary with individuals, and when mating systems are restricted, will tend to become associated with particular families, races, strains, breeds, etc., and it is likely that many differences in fertility that have been observed between such groups are of this nature. It is clear, furthermore, that serious infertility of genetic origin can result when breeders favour phenotypes that are associated with transmissible infertility factors. Examples of this are selection of white coat colour associated with gonad hypoplasia in Swedish Highland cattle (Lagerlöf, 1950, 1951; Lagerlöf & Settergren, 1953), with selection of short legs associated with foetal achondroplasia in Dexter cattle (Crew, 1923), with selection of heavy body type associated with inviable dwarfism in Hereford cattle (Johnson, Harshfield & McCone, 1950), and with selection of hornlessness associated with intersexuality in goats (Laor, Barnea, Angel & Soller, 1962).

LETHAL FACTORS THAT ARISE IN THE SPERMATOGENIC TISSUE

It is important to remember that the word 'genetic' is not synonymous with 'inherited'. The second category of lethal factors, those that arise in the spermatogenic tissue during the lifetime of the male, is indeed likely to be the most important of the three groups listed above and in all probability the majority of lethal factors carried by spermatozoa belong to this class (*see* Hadorn, 1961). These factors may arise spontaneously or be induced by external agencies. In either event they should increase in number with time and could therefore explain the decline in fertility of bulls with age that has already been referred to and has also been observed by other workers (Milk Marketing Board, 1950).

The syndrome of senescence is complex, but a likely fundamental cause of ageing, which may be defined as a progressive loss of functional capacity with time, is a progressive accumulation of mutations in the cells of the body generally. Since each mammalian nucleus contains a very large number of genetic

symbols, probably of the order of a thousand million, it would be surprising if mutations did not arise frequently, particularly when chromosomes are replicating and dividing. Although this thesis cannot be subjected to conventional genetic tests, it is supported by the known incidence of spontaneous heteroploidy in mammalian somatic cells (Beatty, 1951, 1954; Bungenberg de Jong, 1957). Since mutations are almost invariably harmful, it follows that mutated cells will be functionally impaired. The progressive accumulation of mutated cells will lead to progressive loss of functional capacity in the organism as a whole and ultimately to physiological failure and death.

It is a common platitude that germ cells are immortal and do not age. This contention evades many issues. It is known that germ cells mutate and must be subject to natural selection, that most die without issue and that the genotype of the germ cell changes with each diploid generation. It is likely that many of the lethal factors that arise in germ cells are eliminated during gametogenesis—in fact one would expect that the majority of dominant lethal factors that operate at the cell level would be lost at this time. Spontaneous losses of spermatogenic cells are considerable (*see* Bishop & Walton, 1960) and spontaneous losses of oogenic cells are even more remarkable (*see* Ingram, 1962). Nonetheless it is well known that lethal factors of various kinds are carried by germ cells, and it seems reasonable to suppose that they will arise frequently in spermatogenic tissue where cell division proceeds at a fast rate. Heteroploid germ cells are known in a number of mammalian species (Beatty & Fischberg, 1951; Fechtmeier, 1961) non-disjunction of bivalents during meiosis is believed to be a frequent cause of faulty chromosome complement in bull spermatozoa (Melander & Knudsen, 1953; Knudsen, 1956), and abnormal deoxyribonucleic acid content in spermatozoa and spermatogenic cells is associated with infertility in man and the bull (Leuchtenberger, 1960). The accumulation of transmissible lethal factors with time would be expected to give rise to an increasing incidence of embryonic death and hence to increasing infertility, and Sonneborn (1960) cites highly suggestive evidence that the frequency of early foetal death in human beings increases with the age of the male.

Many of the symptoms of senescence can be experimentally induced by ionizing radiation, a potent mutagenic agent. The application of ionizing radiation to the testis not only destroys many of the spermatogenic cells present, but also induces mutations in many that survive (*see* Bishop & Walton, 1960). In man, it is estimated that the present mutation rate would be doubled by a dose of between 30 and 80 r administered to the gonads during the first 30 years of life (Medical Research Council, 1956; National Academy of Sciences, 1956). In mice and other small mammals, matings following irradiation of the testis often result in infertility associated with a high incidence of death among embryos and fetuses (*see* Bishop & Walton, 1960; Hadorn, 1961). This is caused by changes in the nature and arrangement of the genetic material that do not prevent spermatozoa from fertilizing eggs, but do prevent the successful development of zygotes. Some of the semi-sterility resulting from irradiation is known to be caused by induced translocations which may give rise to either viable or inviable genotypes after meiosis and can be transmitted indefinitely through successive generations of viable progeny (*see* Hadorn, 1961).

Effects very similar to those produced by ionizing radiation can also be produced by a large variety of chemical mutagens, some of which, such as nitrogen mustard and triethylenemelamine, are very potent in their effects (Falconer, Slizynski & Auerbach, 1952; Bock & Jackson, 1957; Craig, Fox & Jackson, 1958; Cattanaach & Edwards, 1958). Some of these chemicals appear to provide promising means of oral contraception, but the possible dangers inherent in their use cannot be over-emphasized. Sudden changes of temperature and many therapeutic agents are also known to be mutagenic, and it is conceivable, particularly in view of the exposed position of the testis, that apparently trivial events may underlie many instances of temporary infertility that are reported from time to time. Fortunately, most of the genetic damage inflicted on the testis at any one time is rapidly removed from the reproductive tract, for the stem cells that are responsible for the continual renewal of the spermatogenic epithelium constitute less than 1% of the spermatogenic cells present (*see* Bishop & Walton, 1960). If viable, however, mutated stem cells will continue to repopulate the testis indefinitely.

Finally, in this connexion, it is probable that different genotypes differ in their susceptibility to mutagenesis. This will lead to differences in mutation rate and on the above hypothesis will be associated with differences in the rate of ageing and in the rate of decline in fertility between individuals and between families, races, strains, breeds, etc.

LETHAL FACTORS THAT ARISE IN SPERMATOZOA AFTER THEIR RELEASE FROM THE TESTIS

The nucleus of the spermatozoon is quite unlike that of any other cell and in form is more like a virus than any other biological entity. It consists of closely packed deoxyribonucleic acid bound by an unusual protamine-like nuclear protein (*see* Bishop & Walton, 1960). It appears to play no role in the metabolism of the spermatozoon and to have as its sole function the transmission of paternal genetic material to the egg. In accordance with its peculiar function, the spermatozoon nucleus may be seen as a compact and stable store of genetic codes. It is clear, however, that this nucleus is by no means immutable and that spontaneous and induced changes in its structure can lead to embryonic death.

Mutations in the spermatozoon nucleus are again likely to increase in frequency as a function of time. During the first part of its life within the ductus epididymis, the spermatozoon gradually attains its full functional competence. Later it enters into a state of senescence when its functional capacity becomes progressively diminished. In general, it appears that the ability to contribute to a viable embryo is lost before the ability to fertilize an egg and that fertilizing capacity is in turn lost before the capacity for motility. Young (1931) studied the effects of ageing on the fertility of guinea-pig spermatozoa by causing them to be retained within the ductus epididymis for 20 to 25 days before they were used for insemination. As a result of this treatment, the incidence of non-viable embryos increased from 3.6 to 20%. This observation suggests that infrequent mating by a male could itself result in an increased amount of embryonic death. Crew (1926) studied the effects of ageing on the fertility of fowl spermatozoa stored within the female reproductive tract. Female birds, unlike mammals,

are able to store the spermatozoa of each mating and use these stores to fertilize successive eggs as they pass through the oviduct. When a new mating occurs, the fresh spermatozoa are prepotent over the old ones. Crew (1926) isolated mated female chickens and examined the development of successive eggs. His observations, which have since been confirmed by Nalbandov & Card (1943) and Dharmarajan (1949) show a progressive increase in the incidence of malformed non-viable embryos with increasing age of the spermatozoa. There appear to be no similar observations in mammals, where, in most species, mating and ovulation are closely approximated by limited periods of oestrus behaviour and spermatozoa survive in the female tract for only a short time. In human beings and some other primates, however, mating can occur at any point in the female reproductive cycle, and it is conceivable that fertilization with aged spermatozoa could make a significant contribution to embryonic death in these species. The possibility that mutation may arise in spermatozoa during storage *in vitro* also requires examination. It is well known that the fertility of bull semen stored *in vitro* at 4° C falls by 2 to 6% per day even though the samples appear to contain an adequate number of apparently normal living spermatozoa. It is also known that this rate of decline in fertility differs significantly between samples from different bulls (Hagelberg, 1952; Cembrowicz, 1952; Campbell, 1953). It is likely that part of this loss of fertility is associated with an increase in embryonic death and supporting evidence for this has been obtained by Salisbury, Bratton & Foote (1952a, b), Willett & Ohms (1955), Dzuik (1959) and Dzuik & Henshaw (1958).

The experimental induction of lethal mutations in spermatozoa, both in the ductus epididymis and *in vitro*, has been achieved many times. Snell (1933), for example, irradiated male mice with up to 800 r of X-rays after ligating the ductuli efferentes. Snell found that the longevity of spermatozoa within the epididymis and the capacity of spermatozoa for motility were little affected by irradiation, but matings from treated males resulted in small litters and heavy embryonic mortality. Bruce & Austin (1956) subjected the genital region of male mice to much heavier doses of X-rays and found that spermatozoa could still penetrate eggs when the administered dose was as high as 30,000 r. Syngamy was delayed, however, and further development quickly ceased. Similar results have been obtained after the administration of chemical mutagens to male animals. Thus Jackson and his associates have shown that certain alkylating agents, such as triethylenemelamine and ethylmethanesulphonate, produce almost immediate sterility in male rats, although the spermatozoa retain their motility and are able to reach and penetrate eggs (Jackson, 1960; Jackson, Fox & Craig, 1961).

Interest in the effects of ionizing radiations on spermatozoa *in vitro* was first stimulated by Hertwig's (1911) experiments on the effects of radium emanations on frog spermatozoa. Over the lower part of the dose range, inseminations with treated spermatozoa resulted in an increasing incidence of embryonic death with increasing radiation dosage, but at higher levels this trend was reversed and the incidence of embryonic death declined with increasing dosage. Hertwig explained these remarkable results by postulating that at the lower dose levels the damaged spermatozoon nuclei entered syngamy and produced inviable

embryos, whereas at the higher dose levels the nuclei were so seriously damaged that syngamy was prevented and the activated eggs developed gynogenetically (i.e. without a paternal genetic contribution). The correctness of Hertwig's interpretation has since been confirmed by other workers. Rugh (1939), for example, observed an increasing proportion of embryonic death with increasing dosage of X-rays until virtually all embryos died at a dose of 10,000 r. At higher doses, the incidence of embryonic death again decreased and, at a dose of 50,000 r, 90% of embryos developed as gynogenetic haploids. Similar observations on mammalian spermatozoa show that these too can withstand very high doses of X-rays without any obvious effect on their motility. Insemination with treated spermatozoa again leads to an increasing incidence of embryonic death, but the induction of successful gynogenetic development has not been observed (see Parkes, 1960).

Treatment of spermatozoa with ultra-violet irradiation also leads to embryonic death and gynogenesis in amphibia and to embryonic death in mammals but ultra-violet radiation is much more lethal to spermatozoa than is X-irradiation and high doses lead to fertilization failure. Various chemicals, such as trypanflavine, toluidine blue and nitrogen mustard produce rather similar effects to those of X-rays (see Parkes, 1960).

The experiments considered above illustrate very clearly the extent to which different aspects of biological competence in spermatozoa may be dissociated from one another and the limitations of the conventional approach to the study of semen quality. They also pose the important question of whether any of the common manipulations to which spermatozoa are subjected before artificial insemination or any of the commonly used spermicides are mutagenic. Assurances are often given that there is no risk of mutagenesis from these causes, but these assurances rest solely on the fact that no induced mutations have been observed. This cannot be regarded as satisfactory because dominant mutations are usually lethal and may easily pass unnoticed through embryonic death and because recessive mutations can be readily detected only by special mating systems. The possibility that dominant lethal mutations may be induced in spermatozoa during storage *in vitro* at normal refrigeration temperatures has already been mentioned. There is also suggestive evidence that mutation of this kind may be induced during storage at deep-freeze temperatures and that the loss in fertility that invariably accompanies the deep-freezing of spermatozoa may be associated with an increased incidence of embryonic death (Madden, 1960). This, indeed, would seem to be the only possible explanation for a decline in fertility in the presence of an adequate number of fertile spermatozoa. From a eugenic point of view, the crucial question is whether or not recessive mutations can be induced in spermatozoa and as yet the necessary mating trials to detect this do not appear to have been carried out.

GENERAL CONCLUSIONS

The available evidence suggests that a considerable incidence of embryonic death is a characteristic of reproduction in all mammals. This loss is frequently referred to as 'reproductive wastage' with the implication that it could be avoided. Waste, in the sense of loss without purpose, is however not a feature

of biological systems, for natural selection ensures its continual elimination. It follows, therefore, that embryonic death requires an explanation in terms of biological advantage to the population or species as a whole. The explanation advanced here is that embryonic death provides one of the means of eliminating unfit genotypes at a low biological cost. (An additional suggested means of eliminating unfit genotypes is by selective mortality in gametogenic cells.)

That genetic factors can lead to embryonic death is unquestionable. That many of these are not inherited but arise *de novo* in each generation is also unquestionable. The question that does remain, however, is whether genetic factors can account for a large proportion of the observed embryonic loss. The necessarily tentative opinions of contemporary authorities on mutation would suggest that it can (*see* Hadorn, 1961). Muller (1954), for example, estimates that one third of all human gametes carry a mutation that has arisen in the parent generation and that at least 20% of human embryos die as a result of spontaneous mutations. This incidence, as indicated above, can be greatly increased as a result of environmental influences. To a large extent induced mutations can be avoided, but spontaneous mutation is the price one has to pay for maintaining a desirable degree of plasticity in the genetic material. There is also reason to believe that the inherited load of lethal and incompatible factors may be much higher than was formerly believed (*see* Wallace & Dobzhansky, 1960). One cause of this is exemplified by recent studies of sickle-cell anaemia in human beings (Allison, 1954). The mutant gene that leads to the synthesis of sickle-cell haemoglobin is lethal in the homozygous state, but in certain environments (malarial areas) the heterozygote is at an advantage over the homozygous normal. This situation ensures that the sickle-cell gene is maintained at an unexpectedly high frequency in malarial areas. It also leads to the unsuspected realization that an unfit mutation may be fitter than normal in particular environmental situations and sheds light on the probable nature of heterosis and the biological significance of balanced polymorphism. It follows that part of embryonic death is probably a consequence of the necessity of maintaining genic diversity within the population (*see* Wallace & Dobzhansky, 1960; A. L. Ogden, personal communication, 1963). This leads to yet a further amendment of our concept of fertility, for if fertility depends upon the maintenance of particular genes within the population, it becomes not merely a property of particular mating systems, but a property of the population as a whole.

In the conclusion to his review on prenatal mortality in farm animals, Hanly (1961) writes as follows: "The literature reviewed indicates clearly that the knowledge of the causation of the death of the fertilized ovum and embryo remains incomplete. While numerous factors have been shown to influence embryonic death either by increasing or decreasing it, there is no factor or combination of factors so far investigated, whose control has eliminated it in a group or population of animals. This residual embryonic death, which appears to be relatively constant in amount, particularly in the bovine animal, would seem to have to be accounted for by a more universally active factor than any of those so far investigated." The main purpose of this essay is to suggest the probable nature of this "universally active factor".

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