PATTERNS OF INHERITANCE IN THE DIMENSIONS OF MOUSE SPERMATOZOA

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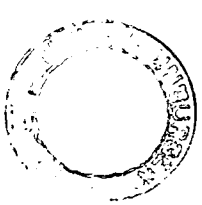

ABSTRACT

Three dimensional characteristics of spermatozoa were studied for their modes of genetic behaviour. The material consisted, at the start, of four highly inbred strains of mice (V, JBT, CBA and C57), and the dimensions were : Head breadth (HB), Head area (HA) and Midpiece length (ML).

After a diallel crossing of these strains, it was observed that the variation between different genotypes was largely due to strong inter-strain differences (used as a measure of additivity of the genes causing strain-specificity). In an alternative statistical analysis, this strain effect was reflected as strong male and female parent effects. There was a significant parental sex effect in ML, meaning that the strains when used as the male parents had different effects than when used as female parents. Mean dominance was observed in HA and ML, and in ML the variation between individual sets of crosses in their degree of dominance was also marked. Other sources of variation were of minor importance. Most of the variation within each genotypic group was attributable to the variation between spermatozoa within preparations (biologically, between spermatozoa within a male). The variations between males within genotypes and between preparations within males were considerably smaller. The within-male variance was not different in crossbred and inbred progeny, indicating an absence of haploid effects (expression of the haploid genome contained in each normal spermatozoon) on spermatozoan dimensions. The data bearing on this important question of haploid effect are probably the most extensive so far available. The HA appeared to be correlated with the body size. CBA and C57 strains

appeared to vary most.

These two strains (CBA and C57) were chosen and the Y chromosome of one strain was put on to the genetic background of the other strain by repeated backcrossing of each reciprocal F_1 male to the female belonging to its mother's strain through several generations. A comparison of backcrosses after six generations of backcrossing (upgrading) to pure strains showed that there was no evidence of a Y chromosome effect. This observation was further supported by the findings obtained in another study where a number of genotypes were produced by crossing the two strains and their F_1s and backcrosses in different ways. The latter experiment, the first of its kind, showed that only additive effects were important in the determination of HA and ML, but HB was likely to be controlled by a maternal effect that was confounded with an X chromosome effect.

In an attempt to isolate the non-chromosomal maternal effect from an X chromosome effect, fertilized eggs were reciprocally transferred from one female to another of a different strain (foster-mother) in the blastula stage. The observations on the resulting progeny showed that the non-chromosomal maternal effects were likely to be of very small magnitude, if they existed.

Thus, the results obtained in this investigation show a very marked genetic control of the dimensional phenotype of spermatozoa. But the different dimensional phenotypes do not follow a single pattern of genetic behaviour common to them all. The idea that the strain specificity of the dimensions is solely due to additivity of the genes controlling them, has been put to test, and it has been shown that the HB behaves differently

from other dimensions. A possible control of the HB by the X chromosome has been demonstrated, and a further exploration of this character has therefore become necessary. For the first time, it has been possible to exclude the Y chromosome completely as a potential source of variation in the quantitative studies on these dimensions of spermatozoa.

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CHAPTER I

INTRODUCTION

Genetic control of gametic characteristics has been widely studied in recent years. The dimensions of spermatozoa, in fixed preparations, exhibit certain characteristic patterns of inheritance. Most of the characteristics show a high degree of genetic control. Coupled with the fact that a spermatozoon is a haploid cell possessing one of numerous possible combinations of genes, this has led to speculation about using the spermatozoan phenotype to predict its breeding value. Thus, depending upon the expression of the haploid genome of the gamete, it may be possible to select spermatozoa according to their genetic content and apply selection at gametic rather than at individual level. The term 'genetics of the gametes' has been used for this line of research (Beatty, 1957a, 1958).

The study of gamete genetics is a study of basic material that carries genes from one generation to the other. Thus, it may have relevance to problems of fertility as well as to assumptions of the respective genetic contribution of the male and the female parents (Beatty, 1961). Any observable effect of the X or the Y chromosome on the haploid gamete would open the possibility of sex control. Eugenic application is also a possibility, by selection of gametes in cases where parents are known to be carriers of certain deleterious genes.

Such studies relate also to another field of enquiry - that of inheritance patterns of the dimensions of various types of somatic cells. If the high degree of genetic determination of the dimensions of germ cells or their organelles applies also to somatic cells, then one could imagine the planning of experiments designed to change the dimensions of somatic

cells or cell organelles in subsequent generations.

Improvement of the economic characters of an animal species involves selection. Whatever method of selection is used at present, it is based on appraisal of an animal's own merit or of his relatives' or of both. One is selecting for a diploid individual. If it becomes possible to select the haploid spermatozoa according to their genetic content (or according to their chromosomal content, as in the case of sex-chromosomes), this may well be used to influence the rate of progress in a breeding programme. If selection is to be made on the haploid cells of the gamete stage, it is essential that the variation in the gamete phenotype be studied. There are several publications concerning the causes and nature of variation in the characteristics of male gametes as reviewed hereunder.

GENETIC EFFECTS IN SPERMATOZOA

Mice

Within the order Rodentia, the shape of the sperm head was shown to vary between species (Retzius, 1909). Friend (1936) observed variations in the mean head length of spermatozoa between males of the same species of rodents (mice, rats). Braden (1956, 1959) found significant differences between inbred strains of mice in shape and the length and the breadth (width) of their spermatozoan head. Later, in an elaborate experiment, marked strain differences were observed in the breadth and area of the head and length and breadth of the midpiece of spermatozoa by Beatty & Sharma (1960). Sharma (1960) extended these studies to F_1 crosses and showed that most of the variation in the gamete morphology was additively genetic in nature and that

there was also an evidence of heterosis (deviation of the crossbred mean from the mid-parental mean) in the dimensions of spermatozoa. Evidence of heterosis has been reported also by Mori (1961), Godowicz & Krzanowska (1966), Krzanowska (1969) and Pant (1972). Recently, it has been shown that if the tobacco mouse (Mus poschiavinus) is crossed with the laboratory mouse, the head area of the sperm in the F_1 actually decreases (Doring, Gropp & Tettenborn, 1972).

Mori (1961) studied the sperm head length of two pure strains and observed differences between the strains as well as between the reciprocally crossbred males. His results suggest a strong male parent effect - the two reciprocal crossbreds being closer to the male parent means. That selection for large and small body size influenced the midpiece length of spermatozoa, was shown by Beatty (1969). Diallel analysis of the sperm dimensions of outbred (Beatty, 1963, 1969) and inbred (Pant, 1972) strains has revealed that the variation between various genetic groups is mainly due to line (additive) effects and that there are both the male and the female parent effects. Male parent effects have been found to be the greater in many cases.

A very high heritability estimated for the midpiece length by Woolley & Beatty (1967) was the basis for a selection experiment continued by Woolley (1970). He selected outbred mice for increase and decrease in the midpiece length of their spermatozoa and obtained a steady response in both directions. The realized heritability (which depends on the extent of the response to mass or individual selection) was around 70 %. In this experiment as well as in that of Beatty (1969), dominance was not important in determining the sperm dimensions. Beatty (1970) has calculated

upper limits of heritability (proportion of components of variance : between strains/(between strains + between litters within strains + between males within litters) which are as follows : head breadth, 0.86; head area, 0.83; midpiece breadth, 0.14; midpiece area, 0.00; midpiece length, 0.74 and 0.86.

Illisson (1968) and Williams, Beatty & Burgoyne (1970) used discriminant functions to combine several measurements on the spermatozoa and reported evidence of heterosis when the two strains with maximum difference in their spermatozoan score were crossed. Illisson (1969) has calculated the heritability of the score, which is about 90 %.

In certain strains of mice, the proportion of morphologically abnormal spermatozoa is exceptionally high. Recently, it has been found that genes located on the Y chromosome affect the proportions of these abnormalities (Krzanowska, 1969; Brozek, 1970). Krzanowska (1969) assumes that there are many genes controlling these proportions, of which at least one is located on the Y chromosome.

In a recent paper (Beatty, 1972) marked strain differences have been reported in the length and width of the sperm acrosome, and it has been observed that the proportions of spermatozoa with a 'notched' posterior border of acrosome are largely strain specific, being 34 % and 76 % in two of the four strains studied.

Rabbits

Beatty (1957b) observed the staining characteristics and the incidence of abnormal spermatozoa of rabbits and found variations between males. Beatty & Napier (1960a) found that the length of the sperm head varied between individuals of a litter, whereas differences between litter

means were small. Beatty & Napier (1960b), in another study on six outbred strains of rabbits, attributed a greater portion of the variation in the length, breadth, area and shape of the head, and proportion of live spermatozoa to highly significant strain differences. They observed that the smaller breed (Netherland dwarf) produced larger headed spermatozoa than the larger breed (Edinburgh AS), and there was evidently no positive correlation between sperm head size and body size.

Napier (1961) estimated the heritabilities of six characteristics of spermatozoa, using the son-sire regression coefficient method. His estimates are as follows :

Characteristics of sperm head	Heritability ± Standard error
length	0.72 ± 0.18
breadth	0.71 ± 0.13
area	0.74 ± 0.15
shape (length/breadth)	0.56 ± 0.13
angular % stained	-0.07 ± 0.21
angular % capless	0.14 ± 0.11

From his results it is clear that mensuration characteristics have very high and enumeration characteristics very low heritabilities.

Bulls

Blake (1945) reported that spermatozoa of different breeds can be identified by looking at the slide under microscope. He, however, did not substantiate his claims by quantitative observations. Venkataswamy & Vedanayagam (1962) reported that breeds differed in the length of the midpiece and tail of their spermatozoa. Breed differences in the dimensional characteristics of three breeds were reported by Mukherjee & Singh

(1965). They observed differences in length, breadth and shape (untransformed length/breadth ratios) of the head and the length of the midpiece of spermatozoa. Marked differences between bulls of one breed in the dimensions of spermatozoa collected during peaks of four seasons were also reported by them (Mukherjee & Singh, 1966). Kuciel (1967) studied spermatozoa of Red Spotted bulls and their progenies, and found significant differences in length, breadth and shape of the sperm head of a bull and its five sons. Differences between bulls and breeds in the mensuration characteristics of their spermatozoa have also been reported by Salisbury & Baker (1966a), Hadi (1967), Kuciel & Frybort (1968), Orlovskii, Mencukova & Mahnac (1968), and Bonadonna & RoyChoudhury (1969). Breed differences have also been recorded in the sperm head dimensions of buffaloes (Pant & Mukherjee, 1973).

A case of genetically determined abnormality of spermatozoa and consequent sterility in Friesian bulls has been reported by Donald & Hancock (1953). This report concerns a defect of the acrosome that arises during development. The defect 'knobbed' is probably controlled by an autosomal recessive gene. That there may be certain other features of sperm morphology that have a genetic basis and affect normal fertility, has been reported by several workers (Hancock & Rollinson, 1949; Jones, 1962; Saacke & Amann, 1966; Blom,1966, 1968; Blom & Birch-Andersen, 1966, 1968). Kuciel & Michalak (1970) have found that primary abnormalities (degenerate forms, head and midpiece abnormalities) are influenced by genetic factors to a much greater extent than the secondary abnormalities (of acrosome and tail). Their findings are based on 10 half-sib groups of bulls and the semen samples have been examined in 4 seasons throughout the year. Salisbury & Baker (1966a)

discovered significant sire differences in such enumerative characteristics of spermatozoan shape as the proportion of normal, pyriform and unevenly stained spermatozoa. They also observed that the proportion of abnormal spermatozoa decreased when lines of inbred cattle were crossed. In view of this heterotic effect, it seems likely that these sire differences were largely due to autosomal genes rather than genes on the sex chromosomes (see Krzanowska (1969) and Brozek (1970), in mice, for a Y chromosome effect). Salisbury & Baker (1966b) have also reported significant differences in the incidence of diploid spermatozoa between sire lines.

Rams

Significant differences between males in their sperm head size have been reported by Kastyak & Pszonka (1966).

Poultry

Breed differences in sperm nuclear size were reported by Kashiwabara & Tanaka (1956). In another study, Kashiwabara (1964) confirmed the previous report, and observed a positive correlation with the erythrocyte nuclear size. This may be an indication that the sperm size behaves as any other body cell, as if it were a general nuclear characteristic. If the haploid genome of the gamete is able to manifest its effect on the sperm phenotype, one would expect a difference in the behaviour of somatic cells and gametes, especially when the animals are not highly inbred.

Drosophila

In Drosophila melanogaster, nucleus length (head length) of spermatozoa varied between males within a culture bottle as also

between spermatozoa within males (Sidhu, 1964). Indirect selection for small body size (a correlated response to selection for small wing cell size) had no influence on the head length of spermatozoa (Beatty & Sidhu, 1967). In a diallel cross, most of the variation in nucleus length was found to be due to dominance interactions of genes; parental line, maternal or reciprocal effects in the nucleus length or in the within male variance were negligible, and there was no evidence of a significant sex-chromosome effect (Beatty & Sidhu, 1967). Polymegaly (existence of distinct length classes of spermatozoa within males) has also been reported by them (Sidhu, 1963; Beatty & Sidhu, 1971) and several other workers (Policansky, 1970; Beatty & Burgoyne, 1971).

Hess & Meyer (1963) and Meyer (1972) have concluded that the mean whole lengths of spermatozoa vary according to the number of Y chromosomes that a male's karyotype contains. Their results have been discussed in a paper by Beatty & Burgoyne (1971), where the latter authors have also given their own results on a number of species of Drosophila. The size classes of spermatozoa have recently been shown to be influenced by the rearing temperature of the flies, and it seems that the number of Y chromosomes alone may not necessarily determine the mean whole lengths of spermatozoa (Upadhyaya, 1971).

ENVIRONMENTAL EFFECTS IN SPERMATOZOA

Technical factors

Sciuchetti (1938) studied variability in the length of the sperm head of bulls and found a greater within than between sample variation. The effect of various non-genetic technical factors was studied by Beatty & Napier (1960a). They reported that head characteristics of rabbit

spermatozoa did not differ between duplicate measurements of the same sperm head, duplicate preparations of slides from the same sample of semen, and measurement of spermatozoa from the vas deferens and of those from the ejaculate. Male means for sperm dimensions remained constant over a considerable part of a male's life. The variation between litter means was very small for the length but comparatively larger for the breadth and area of the sperm head. Most of the dimensional measurements have been made in nigrosin-eosin preparations - a method of differential staining that makes it possible to distinguish between 'live' and 'dead' spermatozoa. To find out if this technique of staining had any effect on the dimensional measurements of spermatozoa, Sharma (1960) made observations on the spermatozoa, firstly, when they were alive and, secondly, after they were fixed but were known to be alive at the time of staining. The estimates of correlation coefficients, calculated from his results by Beatty (1970), are reasonably high and significant. Napier (1961) has shown that the head length of living and fixed 'live' spermatozoa, in nigrosin-eosin preparations, differs by about 1 %. Measurements on living and fixed spermatozoa have also been made by Woolley (1970) in mice.

Kuciel (1967) has reported that the dimensions of bovine sperm heads under a 'depletion test' do not differ between the first and the repeat ejaculates. However, a prolonged sexual rest increases the proportion of spermatozoa with acrosomal deformity. Wells, Awa, Jay & Fancy (1971) have observed that when semen from bulls is collected only once a week, the proportion of spermatozoa with acrosomal defects is three times what it is with 4 collections a week. A month's sexual rest causes an increase of about six to seven fold of the normal minimal proportion.

Age and body weight of animals

Beatty & Mukherjee (1963) have observed that in experiments where age is not an object of study but is randomly present, it need not be taken into consideration for head area and midpiece length of mouse spermatozoa. For head breadth and midpiece area, however, extremes of age should be avoided. If extremes of age exist, they should be approximately randomised. Age must be considered and necessary correction made when the proportion of spermatozoa with normal acrosomes is the object of study. Age effects on the dimensions and abnormalities of spermatozoa have also been reported by Venkataswamy & Vedanayagam (1962), Nagy (1965) and Krzanowska (1972).

In some of the earlier studies in rabbits (Beatty & Napier, 1960b), mice (Beatty, 1963) and Drosophila (Beatty & Sidhu, 1967), body size was found to be unimportant in experiments designed for studying genetic effects on sperm phenotype, spermatozoan size being independent of body size. However, in a more recent paper (Beatty, 1969), it has been observed that if means of the midpiece length of outbred strains of mice are plotted against their body weight, a pattern suggestive of correlation emerges. Mukherjee & Singh (1965, 1966) have also suggested that the dimensions of spermatozoa may depend on the body size of the bulls.

Seasons and related factors

Mukherjee & Singh (1966) and Mishra, Singh & RoyChoudhury (1969) have observed that the dimensions of bull spermatozoa vary according to the season. A comparison of components of variance due to bulls and due to seasons has been made in the former study, and seasonal components, though significant, have been shown to be considerably smaller than those

between bulls. In my experiment on buffaloes (Pant & Mukherjee, 1972), the percentage components of variance, due to bulls, due to seasons and due to other sources respectively, were as follows : head length, 65, 0 and 35; head breadth, 20, 32 and 48; head area, 53, 7 and 40; head shape (untransformed length/breadth ratio), 32, 10 and 58; midpiece length, 59, 0 and 41; midpiece breadth, 4, 3 and 93; midpiece area, 6, 22 and 72; angular percentage of unstained spermatozoa, 5, 39 and 56.

It is known that exposure of males to high environmental temperature causes an increase in the proportion of abnormal spermatozoa (Okauchi, 1963; Rathore, 1969; Smith, 1971). An effect of altered composition of inspired air of males (hyperoxia and hypoxia) on the dimensions of spermatozoa has been shown in mice (Mukherjee & Singh, 1967, 1968a; Kumar & Mukherjee, 1968) and fowl (Mukherjee & Singh, 1968b).

Drugs and processing of semen

The midpiece breadth and area of mouse spermatozoa decrease when the males are injected with drugs like adrenaline and atropine (Mukherjee & Ayyagari, 1969; Ayyagari & Mukherjee, 1970). An effect of storage of semen in different diluents on the dimensions of spermatozoa has been reported by several workers (Mukherjee & Dott, 1960; Rathore & Mukherjee, 1961; Tomar, Pandey & Desai, 1964; Jelam & Nambiar, 1965; Pant & Mukherjee, 1971).

This review of the genetic and environmental effects on the dimensions of spermatozoa leads us to certain conclusions and assumptions. The variation in the dimensions of spermatozoa are influenced, to a remarkable degree, by the genetic constitution of animals. Breeds and strains differ

significantly in their spermatozoan phenotype. Differences between breeds and strains are due to genes which act additively, and among the crosses between inbred strains one can see evidence of dominance. The dimensions are less likely to be influenced by most of the environmental factors like age, body size, litter size and number, techniques of measurements etc. The degree of genetic control differs for different dimensions. Midpiece length seems to be literally free of environmental influence, whereas midpiece area seems to be little affected by genetic factors. Because of their high heritabilities, many other dimensional characteristics could presumably be changed in subsequent generations following mass selection. Parental sex effects, which could be cytoplasmic, environmental or sex-chromosomal in origin, are of importance among the crosses between different lines or strains. Sex chromosomes seem to be involved in certain aspects of sperm morphology.

There are two reviews on the subject of genetics of gametes which include studies on the female gametes in addition to spermatozoa (Beatty, 1961, 1970). The recent review covers dimensional, biochemical, behavioural and other biological attributes of spermatozoa, the disturbed Mendelian segregations in spermatozoa, certain attributes of the egg, and gene action in gametes. Some recent work on the genetics of the spermatozoon is included in the proceedings of an international symposium held at the University of Edinburgh on 16-20 August, 1971. The proceedings (published in 1972; Edited by R.A. Beatty & S. Gluecksohn-Waelsch) include papers on structure and development, metabolism and function, phenogenetics, and immunogenetics of spermatozoa, segregation distortion factors in mice and

Drosophila and certain aspects of germ cell sex and chromosomal control of germ cell development and function.

The design of the present work is based on the above conclusions and assumptions. Three characteristics of mouse spermatozoa - head breadth, head area and midpiece length - are known to be genetically determined to a marked extent and hence have been studied. Two other characteristics, midpiece breadth and area, were not studied as they are known to be mostly influenced by non-genetic factors.

The investigation was planned with a view to studying the patterns of inheritance in the spermatozoan dimensions of mice and to finding out if the sex chromosomes had any influence on the dimensions, which in turn may explain the parental sex effects that have been indicated in previous experiments. For this purpose, a diallel cross was made between four inbred strains of mice. Apart from assessing the magnitudes of types of genetic variability, it identified the two strains with most contrasting characteristics of their spermatozoa. The reciprocal F_1 males of these two strains, were later backcrossed to their mother's strains. Thereafter, in each generation of backcrossing, the two parents were chosen in such a way that the male came from the backcross progeny of previous generation, and the female came from the strain which was used as mother in all previous generations. Thus, genes from the maternal strain gradually replaced all the genes from the hybrid in subsequent generations, except the Y chromosome genes. It was assumed that after six generations of backcrossing in such a way the progenies will contain effectively the Y chromosome of one strain, and the X chromosome and the autosomal genes of the other strain. Using these

backcrosses and the purebreds, a final generation of various kinds of genotypic classes was produced. The aim of such an experiment was to study the extent to which a parental genotype (in terms of autosomal and sex-chromosome linked genes) could influence the sperm dimensions of its progeny. The choice of mouse as an experimental animal is obvious. Quick turnover of generations, as in this species of mammals, was essential for this kind of experiment. The results are described in Chapters that follow this Introduction.

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CHAPTER II

SPERMATOZOAN CHARACTERISTICS IN THE PROGENIES OF A DIALLEL CROSS OF INBRED STRAINS OF MICE

Marked differences between inbred strains in their spermatozoan dimensions are already known (see review - Beatty, 1970). The effects of crossing lines or stocks have been reported by various workers (Sharma, 1960; Mori, 1961; Beatty, 1963, 1969; Illisson, 1969; Krzanowska, 1969; Williams, Beatty & Burgoyne, 1970). The present work concerns a particular kind of breeding method - a diallel cross - applied to four well established strains. A diallel cross is a cross in all possible ways between males and females of a certain number of lines or strains, including reciprocal crosses. Such a crossing gives, in a single generation, much information about the patterns of inheritance. The data also permit one to recognize pairs of strains that differ markedly in their spermatozoan dimensions, as well as reciprocal crosses that differ from one another.

MATERIALS AND METHODS

Stocks

Four inbred strains of mice were used. These were : V (V/Be; initially selected for a high incidence of embryos with abnormal chromosomal complement; Beatty, 1954), JBT (JBT/Jd), CBA (CBA/Fa), and C57 (C57 BL/Fa). All the strains had been under full-sib mating for w ell over 25 generations. Two of these strains, CBA and C57 were chosen because it was known that they differ markedly in their spermatozoan dimensions (Illisson, 1969; Williams, Beatty & Burgoyne, 1970).

Crossing and management of the stocks

Males of each of the four strains were crossed with females of each strain, thus producing 16 kinds of offspring. Each of these 16 kinds constituted a 'cell' of the diallel table. Thus, 4 of the cells contained the purebreds and the remaining 12 the crossbreds. In the 4 purebred cells full-sib pairs were crossed. These animals varied between 44 and 64 days in age at the time the matings were set up. The distribution of age was such that an analysis of variance yielded no significant age differences between the cells. All matings were set up within a period of 2 days with a view to obtaining most of the progeny within a short period of time.

In each cage only one pair was kept. The arrangement of cages in the room was random and their position was frequently changed. The temperature of the room was thermostatically controlled within a range of 21^{o} and 24^{o} C. The whole litter was weaned at 21 days after birth and female progeny were discarded. Freshly weaned males were kept in separate cages, never more than 6 in each cage. It was necessary to medicate mice in some cages where dysentery was present. An antibiotic, terramycin, was mixed in the drinking water for 6 days. However, none of the males was suffering from any apparent disease when it was used for making slides of spermatozoa.

From first litters, 5 males were randomly selected in each cell; they came from 2 to 3 different matings. It is known that the identity of the litter within a strain has no effect on spermatozoan dimensions, the litter component of variance being virtually zero (Beatty & Sharma, 1960; Beatty, 1970). For this reason, litter identity was ignored as a source of

variation in the present experiment. The eighty males in the 16 cells varied between 58 and 63 days in age at the time they were killed. Their age distribution is given in Table 1.

TABLE 1. AVERAGE AND RANGE OF THE AGE (DAYS) AND THE BODY WEIGHT (GMS) OF THE PROGENIES OF A 4 x 4 DIALLEL CROSS BETWEEN INBRED STRAINS OF MICE.

♂ parents		♀ parents: V	JBT	CBA	C57
V	Expt 1 : age	59.8 (58-62)	63.0 (63-63)	60.6 (59-61)	62.0 (61-63)
	Expt 2 : age	123.6 (120-134)	100.2 (94-106)	113.4 (108-121)	121.6 (116-134)
	: body wt	31.52 (29.3-34.0)	43.12 (39.7-45.8)	42.32 (39.5-45.7)	34.32 (32.4-35.7)
JBT	Expt 1 : age	60.0 (60-60)	61.4 (59-62)	59.8 (58-61)	59.8 (59-61)
	Expt 2 : age	120.6 (108-137)	119.8 (116-127)	127.4 (114-137)	123.6 (109-134)
	: body wt	36.22 (34.8-40.1)	35.80 (30.6-40.6)	42.66 (38.5-50.9)	35.96 (31.1-41.5)
CBA	Expt 1 : age	62.2 (62-63)	62.0 (61-63)	59.4 (59-60)	61.2 (59-63)
	Expt 2 : age	115.6 (109-120)	133.8 (133-135)	125.2 (114-137)	122.8 (113-136)
	: body wt	40.22 (35.8-40.9)	40.34 (37.8-45.2)	35.90 (32.1-38.2)	34.86 (30.9-38.1)
C57	Expt 1 : age	61.2 (61-62)	61.8 (61-63)	60.0 (60-60)	62.2 (59-63)
	Expt 2 : age	110.2 (108-118)	122.6 (107-137)	122.0 (108-137)	124.2 (113-137)
	: body wt	38.16 (35.0-43.7)	39.14 (37.7-40.4)	39.12 (38.4-40.6)	29.28 (27.5-30.5)

Preparation of slides

There was no satisfactory method of obtaining spermatozoa from a mouse without killing it. Methods of electroejaculation have been described in mice (Snyder, 1966) and rats (Kalasiewicz & Wolanski, 1970), but the disadvantages of these and other methods have been discussed by Woolley (1970). All the males were killed in a randomized order within a period of two days. Each male was stunned and its neck dislocated. After laparotomy, each vas deferens was excised and the contents squeezed out with the help of forceps into a drop of 0.85 % NaCl in distilled water. The contents were mixed with the help of a blunt and thin glass rod. A minute was allowed for the dispersal of the spermatozoa in the normal saline before a drop of nigrosin-eosin stain (Hancock, 1951) was added. The stain was allowed to act for one minute and smears were then quickly made on two clean grease free slides. The smears were allowed to dry and mounted in DePeX.

On an average, it took about 4 to 6 minutes to prepare the smears after the animal had been killed. All the preparations were made in a controlled environment room where the temperature ranged between 21^{o} and 24^{o} C. As there were eighty males, altogether 160 slides were prepared. These were coded by a colleague, and the code was unavailable to me till all the observations had been completed.

Mensuration techniques

Each slide was projected in a system consisting of an illumination source, a microscrope, a reflecting mirror and a drawing platform. A Gillett and Sibert 'Conference' microscope was used. The microscope was fitted with 115 x objective and 5 x eye piece, and there was a built-in

light source with a quartz-iodide lamp. For obtaining maximum illumination and resolution, immersion oil was used both above and below the slide. An aluminized mirror was fixed on the ceiling, above the microscope, in such a way that the emergent light beam fell perpendicular to the inclined drawing platform. A circular area was drawn in the middle of the drawing platform and the images of the spermatozoa were always drawn within this circle. The magnification was estimated using a N.P.L.-calibrated graticule, serial No. 5170/S. Under this system, a linear magnification of x 3160 was obtained.

Twenty spermatozoa were randomly selected in each slide. Outlines of both the head and the length of the midpiece were drawn on one sheet of paper for each sperm. Stained, malformed or diploid heads were rejected. In some cases normal heads had to be rejected, when the junction of the midpiece with either head or the tail piece was not distinct. Midpiece length was drawn as a fine line along its central axis (Figure 1).

The measurements made on the drawings were : Head breadth, Head area, and Midpiece length. Head breadth was taken as the maximum breadth of the head along a line at right angles to the base line. A vernier callipers was used to measure the head breadth and the readings were taken in 0.01 cm units. A planimeter (HAFF; No. 315) was used to measure the head area and readings were taken in 0.05 cm^2 units. To measure the length of the midpiece, a rotameter (map measurer) was used. Readings were taken in 0.025 cm.units. The rotameter was calibrated at intervals against a standard steel rule at a particular temperature (ca. 20^{o} C) to allow for any wear and tear of the measuring wheel resulting from the constant use of the instrument.

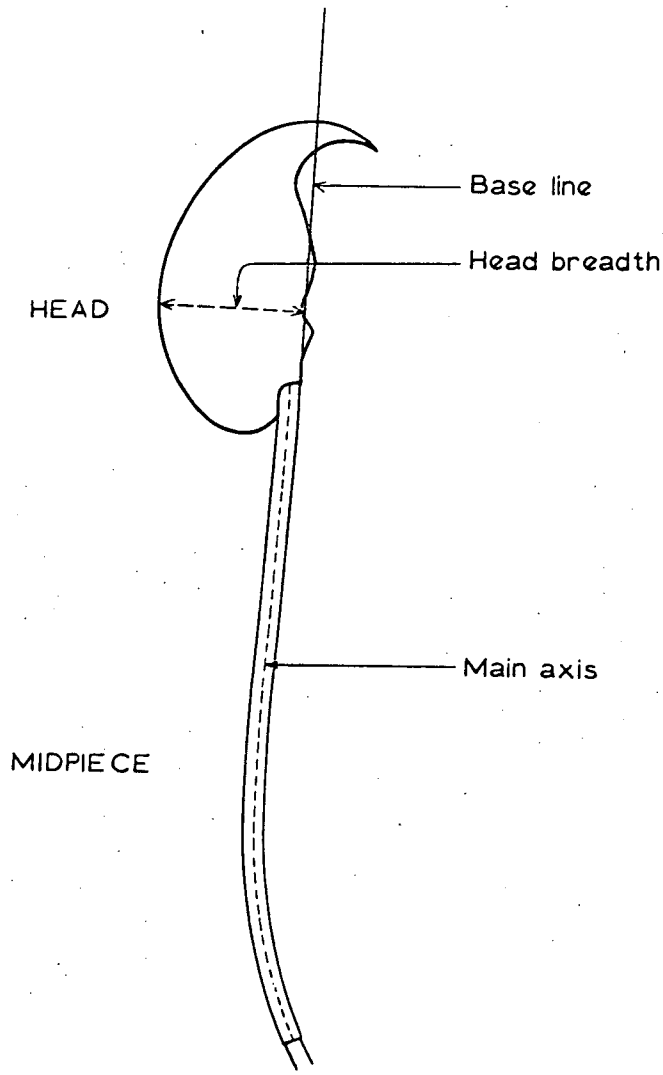


FIGURE 1. Outline of the head and the midpiece of mouse spermatozoon under optical projection.

The measurements were decoded, and statistically analysed according to the principles of Hayman's (1954) and Wearden's (1964) models of diallel analysis. In the analysis, the source of variation was partitioned as shown in Table 3. The analysis was finally converted to μm units. Probability levels have been marked as follows : NS or else no symbol, $P > 0.050$; *, $P = 0.050 - 0.025$; **, $P = 0.025 - 0.010$; ***, $P = 0.010 - 0.005$; ****, $P < 0.005$.

Repeat experiment

The whole experiment was repeated a second time using second litters. Most of the matings, from which the litters were obtained, were those used in the first experiment. The age of the male progeny this time varied between 94 and 137 days at the time they were killed for making the spermatozoan preparations. The age distribution was random between cells, with no significant age differences between cells. The body weights of these mice were recorded just before killing and they are presented, together with the age of the mice, in Table 1. The linear magnification was x 3167. The rest of the procedure was the same as in the first experiment. The two analyses were pooled according to the method described by Hayman (1954).

RESULTS

The means of the spermatozoan characteristics together with the within cell variances are presented in Table 2. Table 2 also shows the body weights of the mice used in the second experiment. The analysis of variance of spermatozoan dimensions and of the body weight is presented in Table 3.

TABLE 2. MEAN DIMENSIONS (MICRON UNITS) AND WITHIN-PREPARATION VARIANCE (FIGURES WITHIN PARENTHESIS, IN MICRON UNITS) OF SPERMATOZOA AND MEAN BODY WEIGHT (GMS) OF THE MALES IN THE 16 CELLS OF A DIALLEL CROSS BETWEEN 4 INBRED STRAINS OF MICE

Characteristics ± Standard error			♀ parents				
			V	JBT	CBA	C57	♂ parent mean
Head breadth ± 0.039	♂ parents	V	3.609 (0.0284)	3.549 (0.0194)	3.622 (0.0229)	3.573 (0.0247)	3.588 (0.0239)
		JBT	3.519 (0.0217)	3.537 (0.0275)	3.641 (0.0229)	3.519 (0.0219)	3.554 (0.0235)
		CBA	3.588 (0.0233)	3.567 (0.0243)	3.682 (0.0257)	3.567 (0.0228)	3.601 (0.0240)
		C57	3.530 (0.0243)	3.532 (0.0234)	3.587 (0.0238)	3.534 (0.0279)	3.546 (0.0248)
	♀ parent mean		3.562 (0.0244)	3.546 (0.0237)	3.633 (0.0238)	3.548 (0.0243)	3.572 (0.0241)
Head area ± 0.397	♂ parents	V	23.250 (2.4465)	24.284 (2.2060)	24.247 (2.2535)	23.308 (2.3950)	23.772 (2.3253)
		JBT	23.780 (2.1310)	24.103 (3.1045)	24.949 (2.3335)	24.224 (2.0680)	24.264 (2.4093)
		CBA	24.040 (2.0695)	24.422 (2.1655)	24.480 (2.3115)	24.232 (1.9240)	24.294 (2.1176)
		C57	22.882 (2.1600)	23.801 (2.0525)	23.874 (2.0415)	22.365 (2.1585)	23.231 (2.1031)
	♀ parent mean		23.488 (2.2018)	24.152 (2.3821)	24.387 (2.2350)	23.532 (2.1364)	23.890 (2.2388)

Midpiece length ± 0.064	♂ parents	V	22.591 (0.2026)	22.341 (0.1633)	22.325 (0.2162)	22.779 (0.1934)	22.509 (0.1939)
		JBT	22.005 (0.2292)	21.626 (0.1800)	21.756 (0.1546)	22.288 (0.1729)	21.919 (0.1842)
		CBA	22.316 (0.2638)	21.822 (0.1641)	21.793 (0.2079)	22.318 (0.2253)	22.062 (0.2153)
		C57	22.792 (0.2104)	22.386 (0.1938)	22.201 (0.2173)	22.605 (0.2698)	22.496 (0.2228)
	♀ parent mean		22.426 (0.2265)	22.044 (0.1753)	22.019 (0.1990)	22.498 (0.2153)	22.247 (0.2040)
Body weight ± 1.366	♂ parents	V	31.52	43.12	42.32	34.32	37.820
		JBT	36.22	35.80	42.66	35.96	37.660
		CBA	40.22	40.34	35.90	34.86	37.830
		C57	38.16	39.14	39.12	29.28	36.435
	♀ parent mean		36.530	39.600	40.000	33.605	37.434

TABLE 3. ANALYSIS OF VARIANCE OF SPERMATOZOAN DIMENSIONS AND OF BODY WEIGHT OF THE PROGENIES OF A 4 x 4 DIALLEL CROSS OF INBRED STRAINS OF MICE.

			Mean squares			
		d.f.	Head breadth (μm)	Head area (μm^2)	Midpiece length (μm)	Body weight (Gms: $\log_{10}$ transfd.)
either	♂ : male parents	3	0.005688*	2.0044****	0.72655****	0.00137
	♀ : female parents	3	0.013419****	1.6154***	0.50179****	0.02524****
or	a : parental effects averaged over sex	3	0.016711****	3.3623****	1.19586****	0.01854****
	c : parental sex effects	3	0.002396	0.2575	0.03247****	0.00806****
	d : parental sex effects within individual sets of reciprocal crosses	3	0.000922	0.0970	0.01440***	0.00263
	b : dominance effects	6	0.001117	0.2888	0.02739****	0.01292****
	b_1 - overall dominance	1	0.003555	1.2372**	0.09205****	0.07350****
	b_2 - dominance of individual lines	3	0.000400	0.1121	0.00675	0.00082
	b_3 - dominance of individual crosses	2	0.000972	0.0797	0.02602****	0.00078

t :	total of diallel	15	0.004452**	0.8589****	0.25950****	0.01101****
B :	block interaction between two sets of diallel	1	0.005247****	0.1472	0.31330****	
Bt :	pooled block interactions	15	0.001499****	0.1577**	0.00285	
M(C) :	males within cells	128[+]	0.000529****	0.0600****	0.00410****	0.00121
P(M) :	preparations within males	160	0.000327****	0.0331****	0.00166****	
S(P) :	spermatozoa within preparations	6080	0.000120	0.0112	0.00102	

[+] d.f. = 64 for body weight

In the analysis, there are broadly 3 main parts : (i) Within cell analysis, (ii) Interactions involving blocks (experiments), and (iii) the Diallel analysis.

Within cells, the males within cells item was tested against preparations within males, and the latter against spermatozoa within preparations. Components of variance are presented in Table 4. The major component of variance is for spermatozoa within preparations. Preparations within males, and males within cells have smaller components.

There are two interactions involving blocks in the analysis. Both were tested against the males within cells item. The block interaction on one degree of freedom represents a general shifting of the overall means of the two experiments. It is most probably a small uncontrollable magnification error. There were two magnifications estimated for the two experiments. These had to be calculated separately because the two experiments were done at different times. Also, the microscope had to be moved for replacement of the illuminating bulb between the two experiments. Such a block interaction was significant for midpiece length and head breadth, but not for head area. It should be mentioned here that midpiece length and head breadth are one-dimensional characteristics, whereas head area is a two-dimensional character. The magnification factor for midpiece length is further influenced by the wear and tear of the measuring wheel that was allowed for by calibration. A small chance error in calibration combined with a similar error in the magnification factor can affect the block interaction.

The pooled interaction on 15 degrees of freedom is an interaction between the sixteen cells and the two experiments. This was not significant for midpiece length and in fact each individual interaction was not significant. This means that the results of the two experiments were

in satisfactory agreement for midpiece length. For this reason, the main effects in the diallel were tested against the males within cells item. In head breadth and head area, however, some of the interactions as well as the pooled interactions were significant. Assuming the strains as fixed effects (the strains were selected), the pooled interaction was considered a valid error for testing the main effects in the diallel.

A brief account of the diallel analysis will now be given, and the results will follow. The 16 cell means in the diallel can be averaged female parent wise or male parent wise (Table 2). Thus we get 4 column means (female parent means) and 4 row means (male parent means). The variation between column means is the female parent effect, and that between the row means the male parent effect, each on 3 degrees of freedom. An alternative kind of analysis (Hayman, 1954; Wearden, 1964) can also be made for these two effects. According to this analysis, row and column means for each of the strains can be averaged separately to give four line means. The variation between these 4 line means gives an effect called parental effect averaged over sex or simply line effect. This is the a item in Hayman's (1954) analysis, and is used for the determination of additive effects and heritabilities. The row and column means can also be subtracted from one another separately for each of the 4 strains, which gives 4 'differences'. The variation between these 4 differences is termed a maternal effect, and is the c item in Hayman's (1954) analysis. In fact, the item c means that the strains when used as male parents do not have the same effect as when used as female parents. It has, therefore, been termed parental sex effect rather than maternal effect in the present experiment.

The item d represents the same type of sex difference as the item c but within individual sets of reciprocal crosses. In other words, item d tells us if differences in the two reciprocal crossbreds of pairs of strains are significantly different from each other. Such variation between differences indicates a sex effect within pairs of strains. Under a purely additive scheme of gene action and with no involvement of sex chromosomes or of non-genetic sex effects, the sex effects of line pairs (d) are not expected to be significant.

The dominance effects can be partitioned into an overall dominance, dominance of individual lines, and dominance of individual crosses. Overall dominance (b_1), on one degree of freedom, represents a general difference between the mean values of all the crossbreds and of all the purebreds. The extent of dominance can, however, be different in different lines and such differences are measured by the item b_2. The dominance of individual crosses is even more specific. Certain crosses may show particularly high dominance where as other pair combinations may show comparatively low or no dominance. Such variations are measured by the item b_3 which has been termed dominance of the individual sets of crosses.

The results are shown in Tables 2 and 3. The parental effect averaged over sex (item a) is highly significant and has the largest mean square in the diallel analysis for all the dimensions of spermatozoa. The male and female parent effects are the next major sources of variation. The male parent effects seem to be larger than the female parent effect in head area and midpiece length, though the comparison is without formal significance. The head breadth, however, seems to be influenced more by female parents than male parents, male parent effects being significant only at the

5 percent level of probability. The other sources of variation in the diallel are not significant in head breadth, and head area shows some evidence of overall dominance. The head area of crossbreds is larger than of the purebreds. Midpiece length, on the other hand, shows a significant effect of all sources except of the item b_2, which means that the dominance contribution of different lines is not differnet, though the different crosses differ in the magnitudes of their dominance. There is also a significant overall dominance in the midpiece length, and midpiece length of crossbreds is greater than of the purebreds. There are also sex differences which are significant for the overall differences among the 4 lines (item c), and also for the two reciprocal crosses of the various pair combinations of strains (item d). Thus, on the whole there are marked male and female parent effects and these effects are due largely to additive gene effects. Other sources of variation in the diallel, though significant, are of minor importance.

The body weight was transformed to $\log_{10}$ values before analysis. It behaved quite differently from the spermatozoan dimensions. There was marked overall dominance, the weights of the crossbreds being distinctly greater than of the purebreds (Table 2). The dominance of lines and of particular crosses did not differ. There was also a marked female parent effect, which seemed (without formal significance) slightly greater than the line effect (a). The parental sex effect (c ; more appropriately a maternal effect in the case of body weight) was also significant, but reciprocal crosses did not show any 'sex' effect (d). The male parent effect in the body weight was not significant.

Within cells, the major component of variance was contributed by the variation between spermatozoa in preparations (Table 4).

TABLE 4. PERCENTAGE COMPONENTS OF VARIANCE IN SPERMATOZOAN DIMENSIONS WITHIN THE CELLS OF A DIALLEL CROSS

Source of variation	Sperm dimensions		
	Head breadth	Head area	Midpiece length
Males	3.7	5.2	5.5
Preparations within males	7.6	8.5	2.9
Spermatozoa within preparations	88.7	86.3	91.6

TABLE 5. ANALYSIS OF VARIANCE OF LOG_{10} OF WITHIN-PREPARATION VARIANCE (MEAN SQUARES)

Source	d.f.	Sperm dimensions					
		Head breadth		Head area		Midpiece length	
		Expt 1	Expt 2	Expt 1	Expt 2	Expt 1	Expt 2
Cells	15	0.0167	0.0482	0.0189	0.0338	0.0793	0.0788
Males within cells	64	0.0237	0.0326	0.0259	0.0390	0.0521	0.0478
Preparations within males	80	0.0180	0.0257	0.0213	0.0329	0.0471	0.0375

An analysis was carried out on the within preparation variances to study if these differed between the 16 cells, and subsequently, if there was a difference between lines, parental sexes, or purebred and crossbred progeny in their within preparation variances. These figures (each on 19 degrees of freedom) were treated as single observations and were transformed to their $\log_{10}$ values before analysis. The results are presented in Table 5. There were no significant differences between cells or between males within cells. The variation between cells was, therefore, not sub-divided.

Using body weights and the spermatozoan dimensions from the second experiment, correlations were calculated between the body weight and the spermatozoan dimensions of the males in the 16 cells of the diallel. An analysis was carried out whereby the total variances and covariances (between body weight and spermatozoan dimensions) were sub-divided into between cell and within cell components, and correlations were determined on cell means and on individual male means within cells. The results are presented in Table 6.

TABLE 6. CORRELATION COEFFICIENTS BETWEEN BODY WEIGHT AND SPERMATOZOAN DIMENSIONS

	Body weight and spermatozoan :		
	Head area	Head breadth	Midpiece length
Overall	0.2890***	0.1093	-0.2896***
Cells	0.6289****	0.1334	-0.3501
Within cells	0.2749**	-0.4289****	-0.1112

TABLE 7. ANALYSIS OF VARIANCE OF REGRESSION OF SPERM HEAD AREA ON BODY WEIGHT OF MICE

Source		d.f.	Mean squares
Cells	Regression	1	80.874***
	Residual	14	8.830****
Error		64	2.242

Analysis of error variance

Error	Regression	1	10.842*
	Residual	63	2.105

Since head area showed positive correlations, an analysis of variance of regression of sperm head area on body weight was carried out and is presented in Table 7. The regression removed a significant amount of the variation between cells and seemed to remove some variation within cells as well.

To find out the extent of differences in the strain means of spermatozoan dimensions, a pair wise comparison of strains was made. The smaller mean was subtracted from the larger mean, and the differences as a percentage of the smaller mean are presented in Table 8. CBA and C57 strains seemed to differ most on an overall basis.

TABLE 8. PERCENTAGE MEAN DIFFERENCES IN THE SPERMATOZOAN DIMENSIONS OF THE FOUR STRAINS OF MICE

Dimensions	V:JBT	V:CBA	V:C57	JBT:CBA	JBT:C57	CBA:C57
Head breadth	2.00	1.98	2.08	3.94	0.08	4.02
Head area	3.54	5.02	3.81	1.54	7.21	8.64
Midpiece length	4.27	3.53	0.07	0.76	4.33	3.60

DISCUSSION

In an experiment of this kind it seems desirable to exclude the possibility of a number of potential sources of variation (other than those considered in the statistical analysis) that may affect the results. On the whole, the effect of such technical or other environmental factors seems to be of minor importance and some publications may be consulted for detailed information (Beatty & Napier, 1960a; Beatty & Sharma, 1960; Beatty, 1970). It will be worthwhile to discuss a few such possibilities to explain why these have been ignored in the present experiment, and detailed discussion of the results will follow.

Environmental effects

For the study of dimensions of spermatozoa, permanent preparations have to be made. Due to several reasons spermatozoa can not be measured with ease and accuracy while they are still alive. Thus, with the conventional methods we try to predict the dimensions of living spermatozoa by studying the fixed one, assuming that the extent of change will not affect the results. Studies have shown that this is true (Beatty & Sharma, 1960; Beatty & Napier, 1960a; Woolley, 1970). The age of the mice used for crossing as well as that of the progeny measured was well controlled. In view of the very small age effects in mice varying greatly in their age (Beatty & Mukherjee, 1963), it is reasonable to suppose that the results of the present experiment are free from any age effects. In general, the dimensions are largely under genetic control and environmental effects are very small. Even then, to eliminate any possible effect of location of mice in the mouse house, the progenies were reared in the same room and their position in the room was frequently changed. The time of preparing all the slides was limited to 36 hours.

All the instruments, saline solution, stain, slides etc., were at the same temperature (ca. 22° C) when the slides were prepared, and thus temperature effects were unlikely. That the mean differences in spermatozoan dimensions much smaller than the resolution power of the microscope can be validly studied has been shown by Beatty & Sharma (1960), and has since been confirmed by different workers. In view of all this, the results on the mean differences between strains and crosses can safely be attributed to real biological differences between them.

Diallel analysis

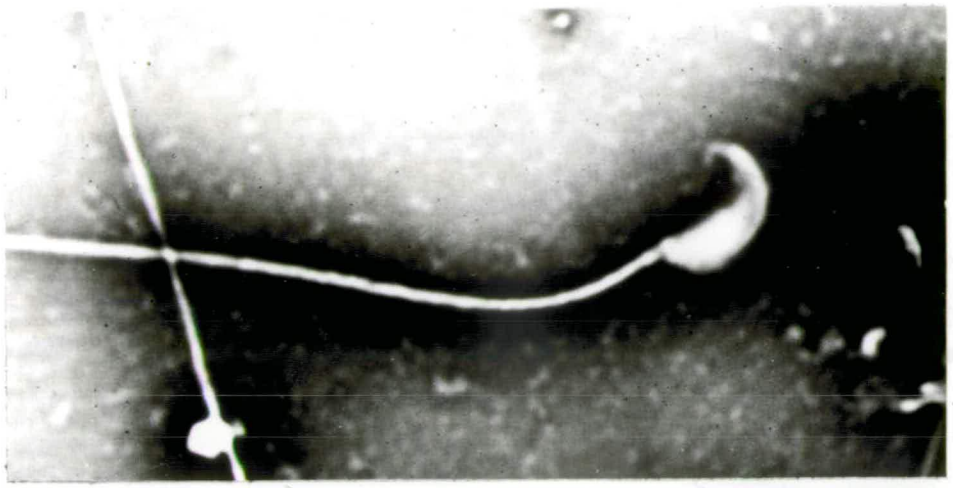
Beatty & Sharma (1960) reported large differences in the spermatozoan dimensions of a number of inbred mouse strains. Later, other workers (Sharma, 1960; Mori, 1961; Beatty, 1963, 1969) crossed inbred or outbred strains of mice and studied the spermatozoan dimensions in the progenies. In the present work, a complete diallel analysis of the dimensions was carried out. In order to minimize the sampling error of each cell mean, 400 spermatozoa (40 from each of the ten males) were measured in each of the 16 cells of the diallel table.

In the diallel analysis, the additive effects (a) seemed to be an important source of variation. This represents the effect of lines and does not take into account the differences between sexes within strains. The lines are genetically distinct groups, and any character showing a large difference between lines can be said to be highly heritable - phenotypic difference corresponding to the genetic difference. If the lines are randomly chosen, such effects will give more or less accurate estimate of a character's degree of genetic determination or heritability, which of course will be an

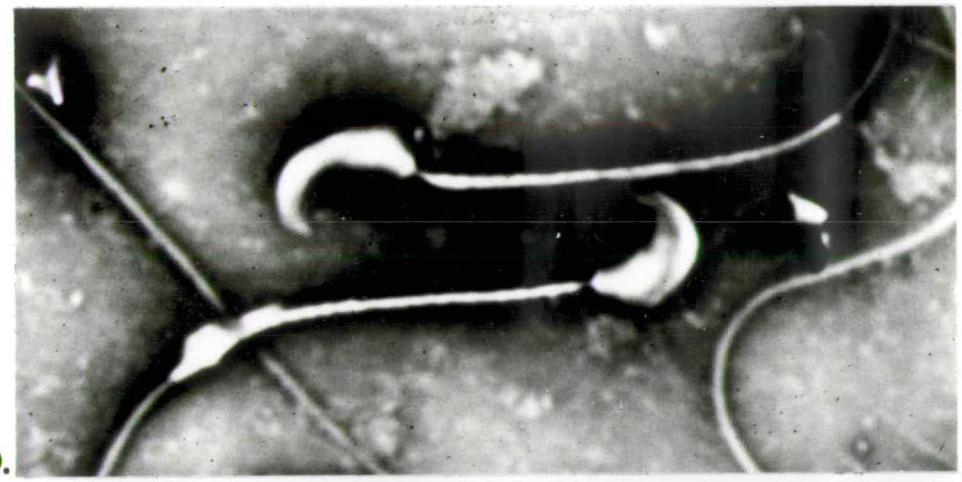
overall estimate for all lines. However, the value of the a item can be increased by selecting lines that differ from each other more widely than unselected lines as a whole. This obviously means elimination of lines from the middle and selection of lines from the two extremes. Such a selection will increase the item a beyond the actual variation between unselected (i.e. randomly selected for diallel crossing) lines, and therefore, any estimate of heritability will be exceptionally high. In the present experiment, at least some of the lines were known to differ in their spermatozoan dimensions and were selected on that basis. It is for this reason that no attempt has been made to estimate heritability and other related genetic parameters.

The large a item suggested that the strains differed in genes that controlled spermatozoan dimensions. Beatty & Sharma (1960) and Illisson (1969) have studied the two strains, CBA and C57, that have been found to differ widely in the present experiment. Illisson's (1969) character W7 is the same as the head breadth in the present experiment and in the paper by Beatty & Sharma (1960). The difference in the head breadth between the two strains in the present experiment was 4.02 percent. Beatty & Sharma (1960) observed a difference of 5.56 percent, and according to Illisson's (1969) observations this difference was as high as 7.19 percent. Her technique, however, was different from the technique used by Beatty & Sharma (1960) and used in the present experiment. C57 strain differs from many other strains in that its sperm heads are typically narrow and short (Beatty & Sharma, 1960; Sharma, 1960; Illisson, 1969). It seems that the proportion of very narrow sperm heads differs between males, and the narrowness of sperm heads varies greatly, sometimes assuming sickle shapes (Plate 1). This increased variation

PLATE 1. Mouse spermatozoa in nigrosin-eosin preparations. Magnification approximately the same in all the four microphotographs. a & b. C57 strain; c & d. CBA strain.



a.



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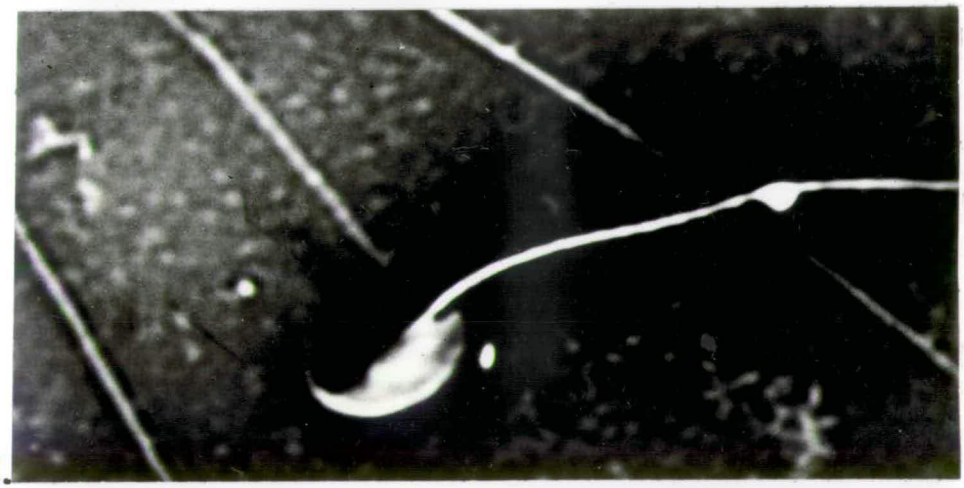
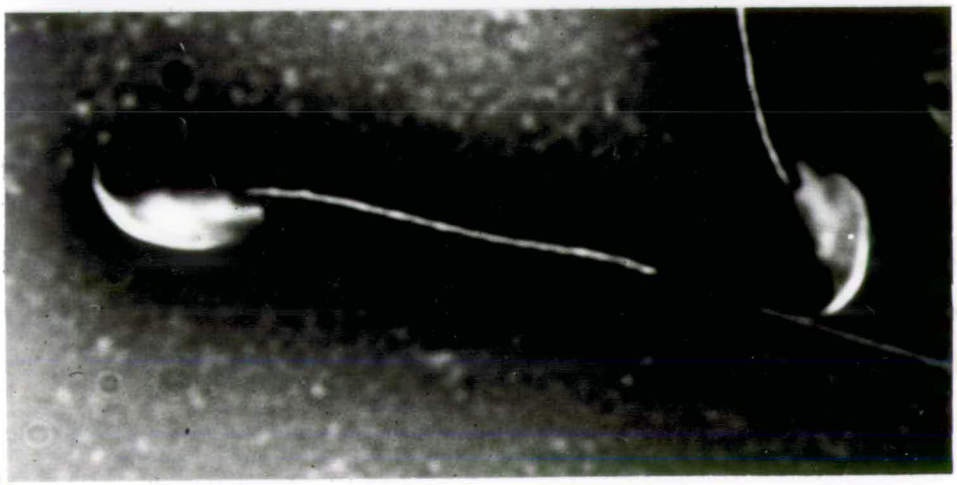


c.



d.

between C57 males may have resulted in unequal male means in the two experiments making the pooled interaction between the two experiments and sources of variation in the diallel significant for head breadth and head area. Such a situation did not exist with the midpiece length and hence the pooled interaction was not significant for midpiece length. Head area and midpiece length differed by 8.64 and 3.6 percent respectively between CBA and C57 strains, and these figures compare favourably (8.48 and 3.21 percent respectively) with Beatty & Sharma's (1960) results.

Male and female parent effects in sperm dimensions have been observed in previous studies of the F_1s. Mori's (1961) results on the head length suggested a very strong male parent effect (sampling unspecified), and he attributed this observation to the dominance of a particular genotype of one of the mouse strains. Beatty (1969) also observed male and female parent effects in head area and head breadth in the hybrid progenies between outbred mouse strains. In the midpiece length, however, he observed only male parent effects. In the present study, male and female parent effects have been observed in all the three dimensions studied. The parental sex effect is not significant in head characteristics, but is significant in midpiece length. A large pooled interaction in the head dimensions is probably responsible for invalidating smaller sources of variation in the diallel. Thus, it seems that the variation between strains (a) in the head characteristics is equally affected by male and female parents. The midpiece length on the other hand may be influenced differently by sexes. On a genetic basis, such differences can be attributed to some genes located on the X or the Y chromosome affecting the length of the midpiece. Beatty (1969) observed male parent effects, but no female parent effects, in the midpiece length and he suggested that genes carried on the Y chromosome could be

responsible for such an effect. Krzanowska (1969) has also shown that the Y chromosome carries factors that affect the proportion of abnormal spermatozoa in certain mouse strains. Differential male and female parent effects might also be produced by extra-nuclear factors carried through the cytoplasm of the egg or of the spermatozoon to the hybrid. Other possibilities include non-genetic maternal effects, either pre-natal (during gestation) or post-natal (suckling, for example).

There are no dominance effects in the head breadth. Sharma (1960) also did not find any dominance in the head breadth. There is a significant dominance in head area and midpiece length, and the two characteristics are larger in the crossbreds than in the purebreds. This is in agreement with the findings of Sharma (1960) and Krzanowska (1969). Heterosis has also been observed in head length of mouse spermatozoa by Mori (1961). Beatty (1969) did not find in outbred mice any dominance effects in the three characteristics that have been studied in the present experiment. He, however, observed mean dominance in two other dimensions, viz. breadth and area of the midpiece, that show very little additive genetic control. Beatty's (1969) stock of mice was outbred and it is possible that a crossing of relatively heterozygous stock did not result in heterosis, as opposed to that observed in the crossing of inbred strains. It may be for this reason that Woolley (1970) also did not find dominance of any appreciable magnitude in his study.

Within-male variance in the spermatozoan dimensions

The variation between spermatozoa in their dimensions within a male is a major source of variation as compared to the variation between male means or preparation means. This has sometime led to the speculation that this may reflect a genetic variation between spermatozoa that

contain a haploid genome. Thus, if the genotype of a spermatozoon can influence its own phenotype, one would expect a poly-modality in the distribution of spermatozoan dimensions, especially if few genes with large effects were controlling the dimensions. In the simplest case, a bimodality may be expected because spermatozoa contain one or the other of the two sex chromosomes. In rabbits, Beatty (1961) failed to find any bimodality in the spermatozoan head length. In crossing experiments, when inbred strains with contrasting sperm dimensions are crossed, one might expect increase in the within male variance in the dimensions of hybrids as compared to purebreds if the segregating genes of the hybrid express themselves in the spermatozoa. Sharma (1960) and Beatty (1969) failed to find such an increase. In the present work too, the within male variance did not differ between purebreds and crossbreds. Illisson (1969) also reported that there was no statistically significant difference in the within male variance between purebreds (SWR and C57) and the F_1. It seems that in such experiments (as in the present work) crossbreds do not show increased variance between spermatozoa. Most of the evidence that has accumulated so far indicates a possible absence of any haploid gene action, and it seems that the genes remain in a dormant state in the spermatozoa. Workers in Drosophila (Muller & Settles, 1927; McCloskey, 1966) also support this view, though there are Segregation Distortion (SD) factors in Drosophila (Peacock & Erickson, 1965; Hartl & Childress, 1972) and the tail-less locus (T-locus) in mice (Braden, 1958, 1960, 1972; Bateman, 1960) that result in non-Mendelian transmission of alleles, but explanation of the SD factors as a haploid effect has mostly been contradicted (Peacock & Erickson, 1965; Peacock, Tokuyasu & Hardy, 1972).

Body weight

The body weight was included in the present study to see if a correlation existed between the body size and spermatozoan dimensions. It was, however, possible to analyse the body weight into various components in the diallel. Most of the variation was due to female parent effects, and in view of the zero male parent effects it seems that pre- and post-natal maternal effects were largely responsible for the adult body size. There were marked additive effects, and it is possible that a genotype that basically controlled the 'mothering ability' of the female, indirectly influenced body size through the mother. There was also a marked mean dominance which has also been reported in outbred strain crossings (Roberts, 1967; Beatty, 1969).

Correlation between body size and spermatozoan dimensions

In an earlier paper, Beatty & Napier (1960b) reported that the smallest breed of rabbits (Netherland dwarf) had the second largest sperm head among several strains studied by them. But, they suggested, the body size and the size of the sperm head were not correlated in any way. In Drosophila (Beatty & Sidhu, 1967) too, selection of flies for large wing size and thereby large body size (being genetically correlated) did not influence spermatozoan head length. A similar attempt was made in mouse strains that had already been selected for large and small body sizes, and in the report (Beatty, 1969) that followed an earlier brief communication (Beatty, 1963) it has been shown that the midpiece length may be positively correlated with body size. This correlation was based on the means of several strains that were studied. It seemed, according to this author, that selection

for large body size resulted in an increase in the length of the midpiece and indirectly in the amount of mitochondria. Mukherjee & Singh (1965, 1966) have explained the breed variations in the bovine sperm dimensions on the basis of body size of the bulls. With this background available, it was considered worthwhile to make a similar study in the present investigation. The strains in the present experiment were highly inbred and had not been selected for body size. Inbred material may present results entirely different from outbred strains used by Beatty (1963, 1969) and other workers.

The correlations between sperm head breadth and body weight were not significant when either individual male means or cell means were taken into consideration. It is assumed, therefore, that head breadth did not depend upon the body weight or on common genotype determining body size and the head breadth of the spermatozoa. Within cells, the correlations may be considered environmental. Between midpiece length and body weight the correlations were negative and significant when individual male means were used. When variances and covariances between midpiece length and body weight were partitioned into cells and within cells components, correlations were not significant. Midpiece length is a highly heritable characteristic, and the absence of correlation based on cell means (which are genetically distinct) does not mean the overall correlation observed was real. At the present state of experimentation, these correlations between head breadth and body weight, and midpiece length and body weight can not be considered of any significance. A large number of males whose age is controlled need to be used and a separate experiment designed.

The head area, however, showed positive and significant

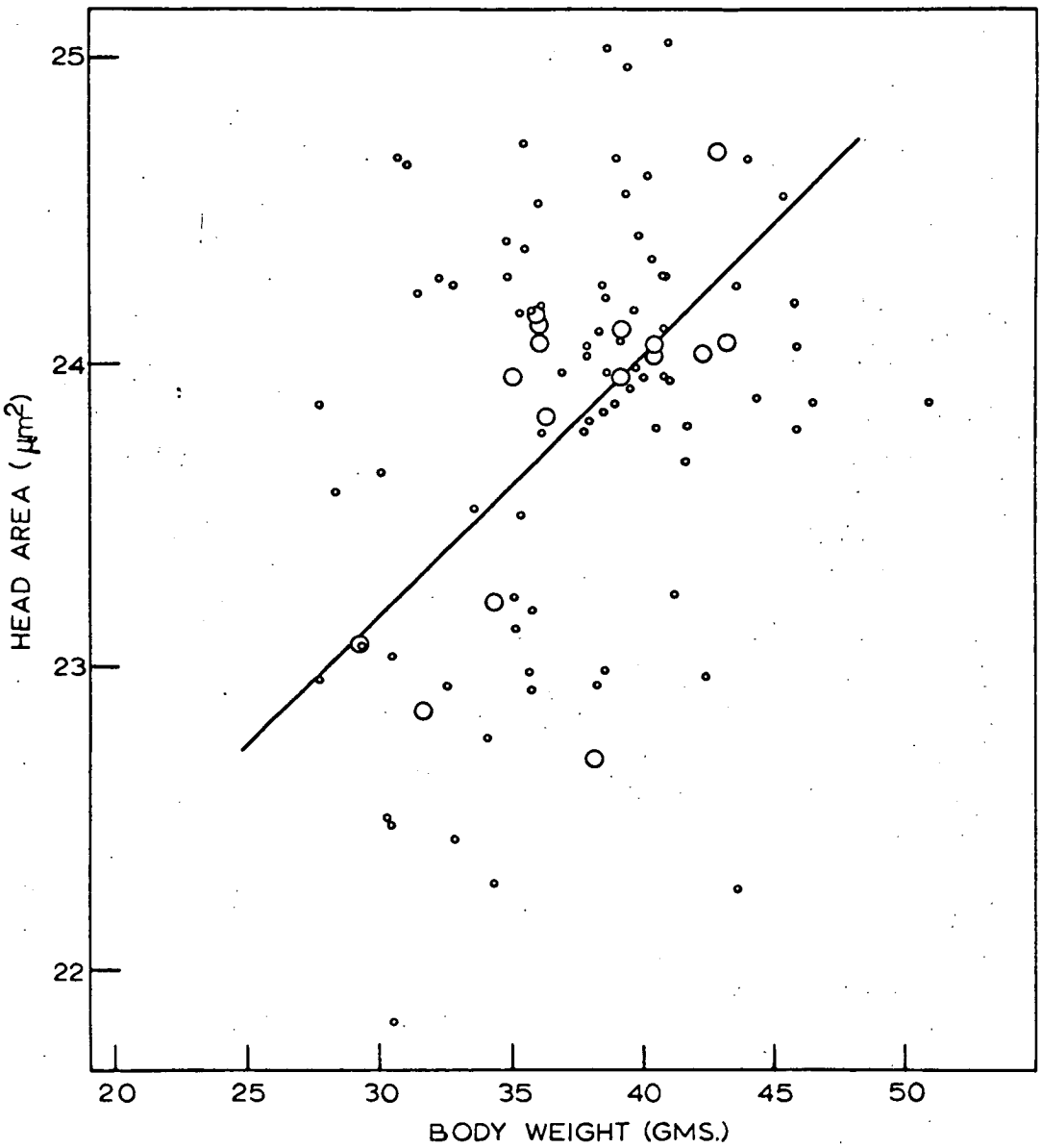


FIGURE 2. Relationship of sperm head area and body weight of mice. Large circles represent 'cell' means and small circles individual male means.

correlation with body weight. The correlations were positive and significant when (i) individual male means for head area together with their body weights were directly used without considering variation between cells, (ii) when these observations were averaged cell-wise to yield 16 pairs of observations and correlations were estimated, and (iii) when the male means were used after the variation between cells had been eliminated in an analysis of variance. The individual male means and cell means have been plotted in Figure 2. A regression coefficient of 0.0875 μm^2 head area per gramme body weight was calculated using the 16 cell means, and the slope is shown in the figure. A positive correlation between sperm head length of sires and birth weight of their progenies was reported by Napier (1961). Rather vaguely, this type of correlation may be explained on the basis of cell size being a general cell/nuclear characteristic (Woolley, 1970). Kashiwabara (1964) found correlations between erythrocyte nuclear size and spermatozoan head length in fowl, indicating a common control of the size/shape of specialized body cells. If this can be further generalised to include other body cells it may well be that a large body size is due to a general increase in the size of many types of body cells including spermatozoa, and this may help explain the correlation that has been obtained in the present study. Mean dominance was observed in body weight and spermatozoan head area but not in head breadth.

SUMMARY

The dimensions of spermatozoa were studied in a diallel cross of four inbred mouse strains (V, JBT, CBA and C57). The dimensions recorded were : Head breadth (HB), Head area (HA) and Midpiece length (ML).

A wide variation existed between the four strains themselves in their spermatozoan dimensions, and this was reflected as a major

source of variation due to additive (line) effects in the diallel analysis. CBA and C57 strains appeared to differ most. In an alternative statistical analysis, the additive effects were shown by both the parents as marked male and female parent effects. Other sources of variation were either of minor importance or insignificant. HA and ML of crossbreds were greater than those of the purebreds. HB did not show this heterotic effect. Within a genotypic class, most of the variation was between spermatozoa within preparations; the variation between duplicate preparations or between males was much smaller. The variation between spermatozoa within preparations was not different in different genotypic classes or between purebreds and crossbreds.

Body weight of the adult males was largely influenced by female parent effects and male parent effects were not significant. There was a marked variation between the 4 strain groups (each group including the purebred strain and its all crosses), indicating additive effects. The body weight of crossbreds was significantly more than of the strains.

The body weight and the HA of the different males appeared to be positively correlated.

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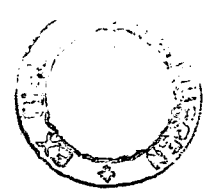

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CHAPTER III

SPERMATOZOAN DIMENSIONS IN CBA AND C57 STRAINS OF MICE, THEIR RECIPROCAL F_1S AND REPEATED RECIPROCAL BACKCROSSES

A male parent effect on the head length of spermatozoa in a 2 x 2 diallel cross of mouse strains was reported by Mori (1961). Beatty (1963, 1969) observed marked male parent effects in diallel crosses between outbred mouse strains. He found that in most cases male parent effects tended to be greater than the female parent effects. He suggested that there may be some type of paternal cytoplasmic inheritance (e.g. mitochondria influencing the length of the midpiece) or else some genes located on the Y chromosome responsible for such effects. Woolley's (1969, 1970) findings have since disfavoured the possibility of a cytoplasmic effect on the midpiece length, presumably also on other sperm dimensions. Krzanowska's (1966, 1969) investigations have shown that the Y chromosome in mice affects the proportion of abnormal spermatozoa and fertility.

If the Y chromosome has any influence on the dimensions of spermatozoa, it should be possible to obtain evidence following a method of continued backcrossing similar to that adopted by Krzanowska (1969). Such a backcrossing will produce two types of mice, the purebreds (produced by full-sib mating of the pure strains as usual) and those with a substituted Y chromosome from another strain. Any difference in the sperm dimensions of these two types may be attributed to the Y chromosome. The present experiment was planned with this aim. It was also considered worthwhile to sample the males produced in each backcross generation to assess the magnitude of changes in their sperm dimensions. For this purpose, the dimensions of purebreds and backcrosses were measured in each backcross generation and compared.

MATERIALS AND METHODS

Purebred CBA and C57 strains and their two reciprocal F_1s were available from the study described in Chapter II. Purebreds were maintained under full-sib mating as usual. The F_1 males obtained by crossing CBA females with C57 males (CBA♀ x C57♂) were backcrossed with the females of CBA strain, and the male progeny obtained in the next and subsequent generations was again backcrossed with CBA females. In the same way, F_1 males obtained by crossing C57 females with CBA males (C57♀ x CBA♂) were backcrossed to C57 females in each generation. In each generation of backcrossing, the two backcross matings and the purebred matings were set up simultaneously so that the progenies were available at nearly the same time, thus eliminating age variations.

Thus, in each generation, there were 4 genotypic classes, two of the purebreds and two of the backcrosses. In the first five generations of backcrossing, 5 males were randomly selected in each class (or cell) per generation. They came from 2 to 3 different matings, and variation between matings was not taken into consideration at any stage for the reasons already explained in Chapter II (see, Beatty & Sharma, 1960). In the sixth generation, 10 males were randomly selected in each of the 4 genotypic classes coming from several matings. The males were killed in a random order, and their age and body weight at the time of killing is presented in Table 1. The sampling was : 1st to Vth generation of backcrossing, 4 cells, 20 males (5 in each cell), 40 preparations (2 in each male), 800 spermatozoa (20 in each preparation); VIth generation of backcrossing, 4 cells, 40 males (10 in each cell), 80 preparations (2 in each male), 800 spermatozoa (10 in each preparation). The procedures of preparing smears, coding, drawing the images of spermatozoa

TABLE 1. MEAN AGE AND BODY WEIGHT (± STANDARD ERROR) OF CBA AND C57 STRAINS OF MICE AND THEIR RECIPROCAL BACKCROSSES IN DIFFERENT GENERATIONS.

Characteristics	Generations	CBA	C57	CBA♀BC	C57♀BC	Overall
Age (Days)	I	100.8 ± 2.94	104.6 ± 0.24	103.2 ± 4.58	104.0 ± 3.42	103.15 ± 3.59
	II	101.4 ± 5.57	106.2 ± 1.20	113.6 ± 0.68	114.2 ± 0.20	108.85 ± 3.21
	III	101.8 ± 1.74	108.2 ± 4.16	105.6 ± 0.24	105.4 ± 0.75	105.25 ± 2.56
	IV	101.8 ± 0.49	101.8 ± 0.73	100.8 ± 0.37	100.0 ± 0.84	101.1 ± 0.71
	V	102.6 ± 1.78	109.4 ± 0.24	100.2 ± 0.92	99.8 ± 0.80	103.0 ± 1.01
	VI	106.1 ± 2.49	104.7 ± 3.48	111.7 ± 0.26	99.6 ± 3.79	105.53 ± 1.54
Body weight (Gms)	I	28.86 ± 1.43	28.06 ± 0.46	33.16 ± 1.60	34.94 ± 0.96	31.255 ± 1.34
	II	31.04 ± 2.84	26.64 ± 0.94	28.78 ± 0.79	28.36 ± 0.77	28.705 ± 1.79
	III	26.94 ± 0.60	26.56 ± 0.89	27.62 ± 0.94	26.84 ± 1.12	26.990 ± 1.02
	IV	25.22 ± 0.86	23.90 ± 0.66	26.10 ± 0.56	22.70 ± 0.53	24.480 ± 0.75
	V	25.04 ± 0.49	25.38 ± 1.27	26.28 ± 1.78	25.44 ± 0.28	25.535 ± 0.53
	VI	31.44 ± 1.32	26.54 ± 0.92	32.08 ± 0.69	28.12 ± 1.10	29.545 ± 0.62

and their measurements were the same as described in Chapter II. The probability levels in the tests of significance have also been marked as in Chapter II.

In describing the backcrosses, the following system has been adopted.

Matings	Symbols used for the progeny	Matings	Symbols used for the progeny
CBA♀ x C57♂	CBA♀C57♂F_1	C57♀ x CBA♂	C57♀CBA♂F_1
CBA♀ x CBA♀C57♂F_1♂	CBA♀BC-I	C57♀ x C57♀CBA♂F_1♂	C57♀BC-I
CBA♀ x CBA♀BC-I♂	CBA♀BC-II	C57♀ x C57♀BC-I♂	C57♀BC-II
CBA♀ x CBA♀BC-II♂	CBA♀BC-III	C57♀ x C57♀BC-II♂	C57♀BC-III
CBA♀ x CBA♀BC-III♂	CBA♀BC-IV	C57♀ x C57♀BC-III♂	C57♀BC-IV
CBA♀ x CBA♀BC-IV♂	CBA♀BC-V	C57♀ x C57♀BC-IV♂	C57♀BC-V
CBA♀ x CBA♀BC-V♂	CBA♀BC-VI	C57♀ x C57♀BC-V♂	C57♀BC-VI

Also, Backcross = BC; First backcross generation = BC-I; Second generation backcross = BC-II; etc.

RESULTS

The means of the spermatozoan dimensions are presented in Table 2a. The means of the F_1 generation were available from the pooled averages in Chapter II. In each generation of backcrossing, the two purebreds were maintained as usual and their sperm dimensions were also measured along with those of the BC. This was necessary because the overall means are likely

TABLE 2a. MEAN SPERM DIMENSIONS OF CBA AND C57 STRAINS OF MICE, THEIR RECIPROCAL F_1S AND RECIPROCAL REPEATED BACKCROSSES (UNADJUSTED MEANS)

Dimensions	Strains or crosses	F_1 generation	Backcross generations					
			I	II	III	IV	V	VI
Head area	CBA♂	24.480	25.774	25.021	24.911	25.427	25.115	24.852
	CBA♀ x C57♂	23.874	24.959	25.139	25.335	25.550	25.506	24.545
	C57♀ x CBA♂	24.232	24.886	23,372	23.782	23.199	23.331	22.954
	C57	22.365	23.848	23.145	23.167	23.099	23.508	22.885
	S.E. on male means	0.245	0.170	0.201	0.307	0.177	0.259	0.141
Head breadth	CBA	3.682	3.797	3.770	3.750	3.797	3.778	3.746
	CBA♀ x C57♂	3.587	3.734	3.747	3.781	3.826	3.829	3.697
	C57♀ x CBA♂	3.568	3.642	3.607	3.613	3.577	3.627	3.580
	C57	3.534	3.660	3.639	3.649	3.631	3.626	3.577
	S.E. on male means	0.023	0.022	0.022	0.031	0.018	0.023	0.015
Midpiece length	CBA	21.793	21.968	21.881	21.790	21.845	21.785	21.858
	CBA♀ x C57♂	22.201	21.953	21.852	21.838	21.817	21.829	21.914
	C57♀ x CBA♂	22.318	22.577	22.529	22.467	22.598	22.511	22.560
	C57	22.605	22.608	22.612	22.507	22.659	22.523	22.492
	S.E. on male means	0.064	0.062	0.062	0.051	0.043	0.034	0.038

to vary a little according to the magnification factor calculated for each experiment (for example, the correlation between linear magnification factors and head areas in the purebreds, in six backcross generations, was −0.8056). For comparing changes in sperm dimensions of F_1 or of different BC generations, therefore, it is necessary to make an assumption, that the means of the purebreds do not vary in different generations or at different times. It has already been observed that the means of the sperm dimensions tend to remain constant throughout a considerable part of a male's life as well as at different times of the same year in laboratory animals (Beatty, 1970). In the present experiment, the largest means were calculated in the BC-I. For the purpose of making comparisons between generations, the means for this generation were taken as an arbitrary standard. The means for all other generations and for the F_1 were adjusted in such a way that there was no difference in the sums of CBA and C57 means in different generations. These means are presented in Table 2b, and are graphically represented in Figure 1. It is clear that the crosses are moving towards the purebreds gradually but steadily in head area and head breadth of their spermatozoa, whereas midpiece length of the BCs has become indistinguishable from the purebreds to which they are being backcrossed after only one generation of backcrossing and has remained so in all subsequent generations. The difference between the two purebred strain means has remained more or less the same, indicating constancy of the results. The data were subjected to analysis of variance presented in Tables 3a, 3b and 3c. The variation between the 4 cells (3 degrees of freedom) was partitioned into (i) a purebred vs. crossbred mean comparison (b_1 item in Hayman's, 1954, analysis), (ii) variation

TABLE 2b. MEAN SPERM DIMENSIONS OF CBA AND C57 STRAINS OF MICE, THEIR RECIPROCAL F_1S AND RECIPROCAL REPEATED BACKCROSSES (ADJUSTED TO 1ST BACKCROSS GENERATION)

Dimensions	Strains or crosses	F_1 generation	Backcross generations					
			I	II	III	IV	V	VI
Head area	CBA	25.931	25.774	25.777	25.711	26.001	25.631	25.833
	CBA♀ x C57♂	25.289	24.959	25.899	26.149	26.127	26.030	25.514
	C57♀ x CBA♂	25.668	24.886	24.079	24.546	23.723	23.810	23.860
	C57	23.691	23.848	23.845	23.911	23.621	23.991	23.789
Head breadth	CBA	3.805	3.797	3.794	3.779	3.812	3.805	3.815
	CBA♀ x C57♂	3.707	3.734	3.771	3.811	3.841	3.856	3.765
	C57♀ x CBA♂	3.687	3.642	3.630	3.641	3.591	3.653	3.646
	C57	3.652	3.660	3.663	3.678	3.645	3.652	3.642
Midpiece length	CBA	21.880	21.968	21.922	21.927	21.880	21.917	21.969
	CBA♀ x C57♂	22.290	21.953	21.893	21.976	21.852	21.961	22.026
	C57♀ x CBA♂	22.407	22.577	22.571	22.609	22.635	22.647	22.675
	C57	22.696	22.608	22.654	22.649	22.696	22.659	22.607

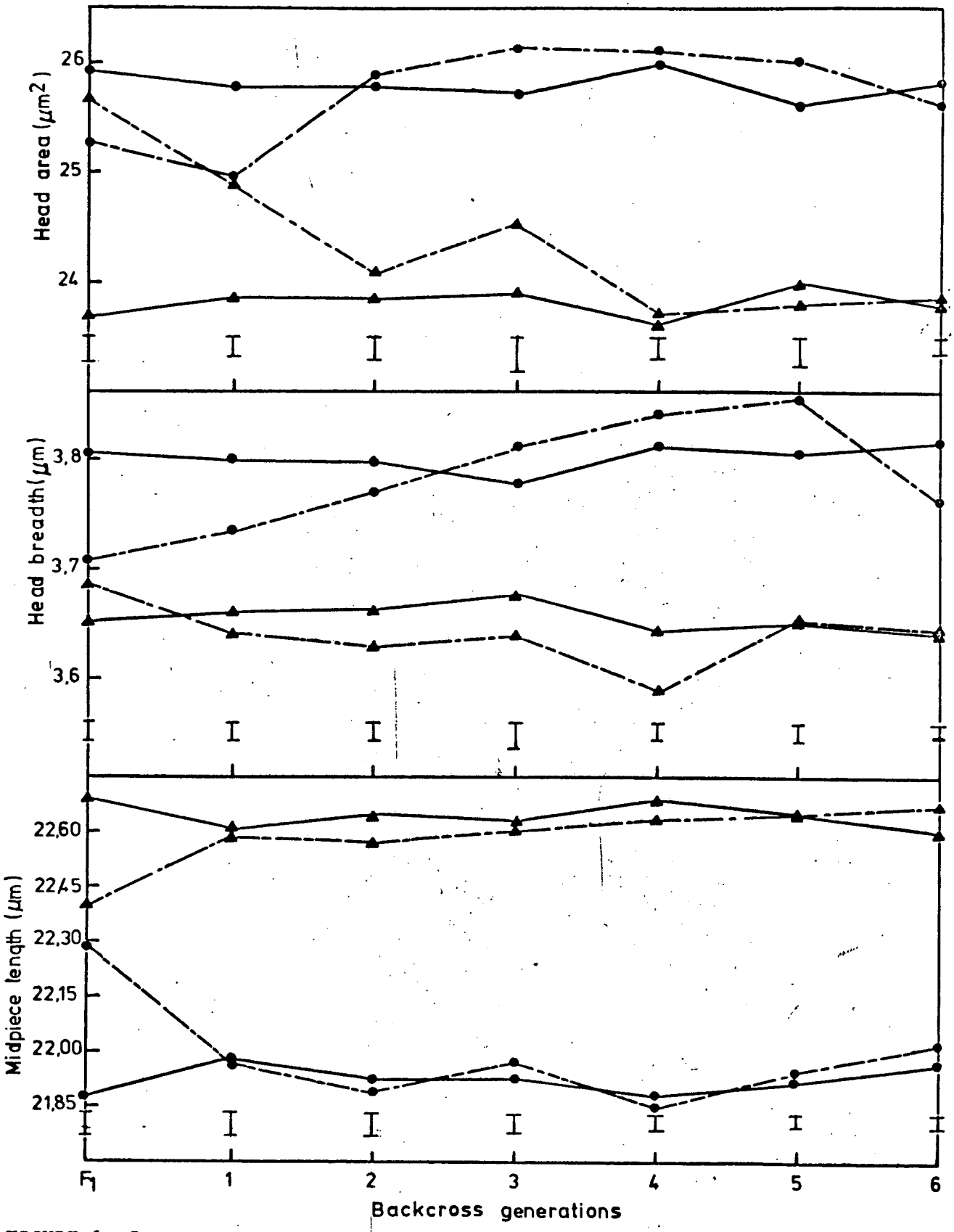

FIGURE 1. Spermatozoan dimensions of CBA and C57 strains of mice and of their repeated reciprocal backcrosses. (Continuous line with dots, CBA; Continuous line with triangles, C57; Broken line with dots, CBA♀BC; Broken line with triangles, C57♀BC.) The perpendicular lines at the bottom of each graph represent one standard error estimated on the male means.

TABLE 3a. ANALYSIS OF VARIANCE OF SPERM HEAD AREA IN DIFFERENT GENERATIONS OF BACKCROSSING (MEAN SQUARES, IN μm^2 UNITS, ON 1 CELL BASIS)

Source of variation		d.f.	Backcross generations						
			I	II	III	IV	V	d.f.	VI
either	♂	1	0.858235 ****	0.002991	0.009193	0.000142	0.080142	1	0.035483
	♀	1	0.998720 ****	3.316733 ****	2.717190 ****	5.473232 ****	3.576127 ****	1	3.164592 ****
or	a	1	1.854295	1.759463 ****	1.521239 ****	2.708786 ****	1.291851 ****	1	1.935135 ****
	c	1	0.002661	1.560261	1.205144	2.764588	2.364747	1	1.264941
Purebred vs. Backcross	(b_1)	1	0.012412	0.029801	0.270098	0.012390	0.011431	1	0.014211
Cells		3	0.623123 ****	1.116508 ****	0.998827 ****	1.828588 ****	1.222676 ****	3	1.071429 ****
Males within cells		16	0.028756	0.040325	0.094418 ****	0.031313	0.066880 ****	36	0.019755
Preparations within males		20	0.016312	0.030057 ****	0.020885 ****	0.020051 ****	0.014887 **	40	0.012248
Spermatozoa within preparations		760	0.012005	0.008713	0.009431	0.008934	0.008136	720	0.009374

TABLE 3b. ANALYSIS OF VARIANCE OF SPERM HEAD BREADTH IN DIFFERENT GENERATIONS OF BACKCROSSING (MEAN SQUARES, IN μm UNITS, ON 1 CELL BASIS)

Source of variation		d.f.	Backcross generations						
			I	II	III	IV	V	d.f.	VI
either	♂	1	0.000502	0.000020	0.001147	0.001706 *	0.000646	1	0.000672
			****	****	****	****	****		****
	♀	1	0.013072	0.018364	0.018209	0.043167	0.031218	1	0.020552
			****	****	*	****	****		****
or	a	1	0.009348	0.008582	0.005108	0.013854	0.114398	1	0.014329
			***	****	****	****	****		****
	c	1	0.004226	0.009803	0.014248	0.031020	0.020424	1	0.006895
Purebred vs. Backcross	(b_1)	1	0.001612	0.000787	0.000005	0.000152	0.000676	1	0.000526
			****	****	****	****	****		****
Cells		3	0.005062	0.006390	0.006454	0.015011	0.010847	3	0.007250
			*		****	*			
Males within cells		16	0.000481	0.000493	0.000930	0.000306	0.000545	36	0.000211
				****	*				
Preparations within males		20	0.000200	0.000285	0.000197	0.000108	0.000099	40	0.000158
Spermatozoa within preparations		760	0.000141	0.000135	0.000117	0.000130	0.000115	720	0.000120

TABLE 3c. ANALYSIS OF VARIANCE OF SPERM MIDPIECE LENGTH IN DIFFERENT GENERATIONS OF BACKCROSSING (MEAN SQUARES, IN μm UNITS, ON 1 CELL BASIS)

Source of variation		d.f.	Backcross generations						
			I	II	III	IV	V	d.f.	VI
either	♂	1	0.000069 ****	0.000717 ****	0.001878 ****	0.000261 ****	0.000773 ****	1	0.00041 ****
	♀	1	0.399234 ****	0.494855 ****	0.452902 ****	0.635467 ****	0.503710 ****	1	0.409286 ****
or	a	1	0.204883 ****	0.266625 ****	0.256550 ****	0.330750 ****	0.271968 ****	1	0.200566 ****
	c	1	0.194420	0.228948	0.198230	0.304979	0.232514	1	0.208761
Purebred vs. Backcross	(b_1)	1	0.000510 ****	0.003161 ****	0.000017 ****	0.001953 ****	0.000254 ****	1	0.003833 ****
Cells		3	0.133271	0.166244	0.151599	0.212561	0.168245	3	0.413161
Males within cells		16	0.003894 ****	0.003829 **	0.002551 *	0.001886 ***	0.001146 **	36	0.001477
Preparations within males		20	0.002937	0.001671	0.000976	0.001420	0.000540	40	0.001114
Spermatozoa within preparations		760	0.001078	0.000899	0.000801	0.000716	0.000732	720	0.000818

between male-parent-wise means, and (iii) variation between female-parent-wise means. An alternative analysis for (ii) and (iii) was done, total variation due to (ii) and (iii) being repartitioned into (iv) a parental effect averaged over sex (Hayman's a item) and (v) parental sex effect (c item in Hayman's analysis), each on one degree of freedom. This analysis has also been carried out in Chapter II, though its meaning in the present chapter is different from that in the previous chapter. In such experiments, where backcrossing is being carried out by using females of the pure strains, one would expect the male parent effects to be significant if the Y chromosome had a marked effect. Where the degree of a Y chromosome influence is small, its expression will depend on the amount of additive genetic variation that is associated with the phenotype of the character, since large additive effects will tend to push the means of the backcrosses towards the female parent means in each BC, thus counteracting any tendency of the Y chromosome to push the means towards the male parent means (whose Y chromosome the backcrosses contain). In the present experiment, the additive effects are large and the females have been used for upgrading the BCs. It is, therefore, the female parent effect that is significant in the present experiment, and there is no evidence of a male parent effect (and thereby of a Y chromosome effect). The differential effect of the two sexes is expressed by the item c, which is significant in all the generations. The difference between overall means for the BC progenies and that for the purebreds was not significant in any of the BC generations for any of the dimensions, thus indicating a complete absence of any effect resulting from crossing of highly inbred strains. Such an effect (b_1: dominance) was observed in the F_1 generation (Chapter II).

Within each cell, the major component of variance was due to spermatozoa within preparations; preparations within males, and males within cells contributed considerably smaller variation. The percentage components of variance due to these factors are presented in Table 4.

TABLE 4. PERCENTAGE VARIANCE COMPONENTS IN THE WITHIN CELL ANALYSIS OF VARIANCE.

Dimensions	Generations of backcrossing	Males within cells	Preparations within males	Spermatozoa within Preparations
Head Area	I	4.8	1.7	93.5
	II	2.6	10.6	86.8
	III	15.5	4.8	79.7
	IV	2.9	5.7	91.4
	V	13.3	3.4	83.3
	VI	3.7	2.9	93.4
Head breadth	I	4.6	2.0	93.4
	II	3.6	5.1	91.3
	III	13.2	2.9	83.9
	IV	3.7	-0.8	97.1
	V	8.9	-0.6	91.8
	VI	2.0	2.6	95.4
Midpiece length	I	2.0	7.8	90.2
	II	5.5	3.9	90.6
	III	4.7	1.0	94.3
	IV	1.5	4.6	93.9
	V	2.1	-1.3	99.2
	VI	2.1	3.4	94.5

(The small "negative" component may be equated with zero)

DISCUSSION

There are only rare phenotypic manifestations in the mouse for which a definite Y chromosome effect has been discovered. Apart

from its function as a strong male sex determiner (Welshons & Russell, 1959), it has been found to contain the histocompatibility antigen gene that is responsible for the rejection of all male skin grafts by the females (Eichwald & Silmser, 1955; Hauschka & Holdridge, 1962; see also, Dronamraju, 1965, for the Y chromosome function in other species). Krzanowska (1969; 1972) provided the first evidence of a definite Y chromosome effect on certain aspects of sperm morphology and function in mice. In an earlier study, she (1966) had discovered that in one of the mouse strains (KE), the proportion of abnormal spermatozoa was exceptionally high (17 %). She found that crossing this strain with a normal strain (KP), which had an abnormal sperm incidence of 6 %, resulted in F_1s with very low proportions of abnormal spermatozoa. Later (1969) she crossed the KE strain with another strain, CBA (abnormality, 5.9 %), and observed a similar 'heterotic' effect. In the subsequent reciprocal backcrosses that she carried out to replace the Y chromosome in the background of another strain's genotype, she observed a reciprocal difference which depended upon the source of the Y chromosome. She concluded that the sperm head abnormality was controlled by several genes of which at least one was located on the Y chromosome.

In the present experiment, two strains with dimensionally different spermatozoa were crossed and backcrossed to see if a Y chromosome controlled situation could be found. It became nearly certain that such a situation did not exist, at least in the strain combination I have used. It seems that primarily additive effects are concerned in the dimensions of sperm head, as expected in view of the earlier findings of a high additive genetic determination of spermatozoa in mice (Illisson, 1969) and in rabbits (Napier, 1961). It seems that the BCs are actually identical with purebreds in the

second and subsequent BC generations (Figure 1), and continued backcrossing has only repeated the results observed in BC-II. Krzanowska (1969) also opined that there may not be any difference in the sperm head of BCs and purebreds in similar experiments. It is worth noting that in inbred strains, Illisson (1969) failed to find a paternal effect in the head shape of spermatozoa, though she did find a strong maternal effect.

The midpiece length in the present experiment behaved slightly differently from head measurements. Instead of following a gradual trend towards the purebreds, it was actually identical with the purebreds in the very first generation of backcrossing (Figure 1).

All these observations show that in the present work there is no Y chromosome effect. The behaviour of the midpiece in the first generation of backcrossing may be a purely additive effect. Presumably, a limited number of genes with relatively marked effects have caused the immediate shifts in the BC-I generation. Illisson (1969) has estimated, from an analysis of variance of the F_2 generation of crosses between C57 and SWR strains, that there are a minimum of two factors that affect the head shape of spermatozoa, and a similar situation can be envisaged for the midpiece length in the present experiment. The following observations show why a purely additive effect is more likely. The papers of Beatty & Sharma (1960) and Sharma (1960) revealed relatively large strain differences in midpiece length, and variation in the F_1 was mostly additive in nature, though heterosis was also observed. Woolley (1970) found a very high heritability of the length of the midpiece (0.76 and 0.88) and observed that the effect of environmental factors and of dominance was very small. Beatty (1970) has calculated upper limits of heritability from differences between inbred

mouse strains, and his estimates for midpiece length are 0.74 and 0.86, which are very close to Woolley's (1970) estimates. The other possible explanations for such a shift in the midpiece length in BC-I are unlikely for reasons that are described hereunder. A 'maternal effect' (not associated with the X chromosome) seems unlikely for two reasons, (i) a maternal effect of the same magnitude as observed in the BC-I should also have been observed in the F_1, and (ii) no effect was noticed in an experiment (described later in Chapter IV), where blastocysts were transferred from one strain to another to see if the development of blastocysts within the uterus of another strain's mother and consequent post-natal maternal environment had any effect on the midpiece length. The possibility of a purely X chromosome effect can also be excluded as its magnitude should remain the same in the F_1: and this is not so. However, one might think that there is a possibility of some interaction between the X chromosome and some of the autosomal genes. Such an interaction may not have taken place in the F_1 as the minimum number of autosomal genes did not reach a particular 'threshold' that was reached after one generation of backcrossing (75:25 grade), and any further addition of such autosomal genes did not produce any increased effect. Another possible reason may be presumed on the basis of some kind of effect that a grandparent's X chromosome might have on the sperm dimensions. But the results obtained in another experiment (Chapter V) show that this is not so. It seems that other kinds of experiments will be necessary, but in the mean time it may be assumed that, except for additive gene action, no other forms of gene action are involved in determining the midpiece length.

SUMMARY

Two inbred strains of mice, CBA and C57, were crossed and backcrossed for six generations in such a way that in every backcross (BC) generation, the male parent was the crossbred (F_1 or a BC) and the female belonged to the strain from which the mother of the male came. The purpose was to put the Y chromosome of one strain into the genetic background of another strain. Three dimensions of spermatozoa, area and breadth of the head and length of the midpiece, were studied and compared with those of the purebreds in each generation. All the dimensions moved towards the maternal strains, and after only two generations of backcrossing were nearly indistinguishable from those of the purebreds. Midpiece length of BCs resembled those of the purebred mother's strains after only one generation of backcrossing. Strong additive effects are confirmed and an effect attributed to the strain-specificity of the Y chromosome is excluded.

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CHAPTER IV

POST-FERTILIZATION MATERNAL EFFECTS ON SPERM DIMENSIONS OF INBRED MOUSE STRAINS

In experiments, where mouse strains have been crossed with a view to studying the sperm dimensions of the hybrids, marked male and female parent effects have been observed (Beatty, 1969; Pant, 1972). Sharma (1960) carried out one-way crosses between several inbred strains of mice and some of his crosses resemble their mother's strain very closely in sperm dimensions. Illisson (1969) demonstrated a significant maternal effect on the head shape of spermatozoa and suggested that such an effect might be due to a permanent influence on the indifferent germ cells or Sertoli cells of the embryos. Beatty (1969) has discussed several other possible sources that may be responsible for differential male and female parent effects.

The present experiment was planned to study the possible effects of maternal environment resulting from the development of an embryo in the uterus and consequent post-natal maternal effect on the sperm dimensions of the progeny. The other possible sources of maternal effects, such as those due to genes located on the X chromosome or due to factors being carried through the cytoplasm of the egg (Beatty, 1969), have therefore been excluded from the 'maternal effect' that is being studied.

MATERIALS AND METHODS

For the experiment, two inbred strains of mice, V and JBT, were available. It was known, from a diallel cross between these two and other strains (Chapter II), that differences in sperm dimensions exist between the strains themselves as well as between their two reciprocal

crosses (Pant, 1972, a brief report).

There were 4 groups that will be called 'cells'. The two purebreds formed two of the cells. In these two cells, males born from purebred females, in the normal way, were used. The third cell contained mice that were taken out of the V females in the blastula stage and transferred into the uteri of JBT females. Similarly, JBT embryos transferred to V females formed the fourth cell. The technique of egg transfer has been described by McLaren & Michie (1956) and Bowman & McLaren (1970). The transfers were kindly performed by Mr. P.S. Burgoyne.

The male progeny were weaned at 21 days after birth. In each cell, one male was randomly selected. It is known that the variation in sperm dimensions between males within inbred strains is virtually zero (Beatty & Sharma, 1960; Beatty, 1970). The 4 males from the 4 cells were killed in a random order for making spermatozoal preparations. Their age at the time of killing varied between 81 and 88 days, and no effect of such a small age variation on sperm dimensions is anticipated (Beatty & Mukherjee, 1963). From each male, 5 nigrosin-eosin slides were made by the method of Hancock (1951) and mounted in DePeX. The slides were coded before examination. In each slide, ten spermatozoa were randomly selected and outline drawings of their heads and midpieces were made on sheets of paper, using a separate sheet for each sperm, under a projection microscope at a linear magnification of x 6144 (Woolley & Beatty, 1967). The characteristics measured were: head breadth, head area and midpiece length. The whole technique has been described by Beatty & Sharma (1960) and also in Chapter II.

RESULTS

In the statistical analysis, the variation between the 4 cell means (on 3 d.f.) was divided into two main factors, (i) between the two genotypes, and (ii) between native and transplanted males. The remaining portion was termed a genotype x transplantation interaction (on the remaining 1 d.f.). Within cells, the variation was partitioned hierarchically as follows : Preparations within cells, P(C); Spermatozoa within preparations, S(P). The item P(C) was used as error to test the main effects between cells and itself was tested against S(P). Thus this analysis combines the effects of transplantation as a technique and post-transplantation maternal effects as one source of variation. The results of the analysis are presented in Table 1, and the cells means are presented in Table 2.

TABLE 1. ANALYSIS OF VARIANCE OF SPERMATOZOAN DIMENSIONS IN μm UNITS OF V AND JBT STRAINS OF MICE

Source	d.f.	Mean squares		
		Head breadth	Head area	Midpiece length
Transplantation plus host mother	1	0.0007039	0.0667	0.01483
Genotypes	1	0.0008488	**** 1.5935	**** 1.02851
Transplantation x Genotypes	1	0.0000045	0.0036	0.00013
Preparations within cells	16	0.0004951	0.0433	0.00530
Spermatozoa within preparations	180	0.0005083	0.0448	0.00329

****, $P < 0.005$

TABLE 2. MEAN SPERM DIMENSIONS OF JBT AND V MALES BORN WITHOUT TRANSPLANTATION (NATIVE) AND AFTER TRANSPLANTATION TO THE OTHER STRAIN FEMALE

Dimensions ± S.E.	Genotype of males	Native	Transplanted	Overall genotype means
Head breadth (μm) ± 0.022	JBT	3.681	3.705	3.693
	V	3.708	3.736	3.722
Head area (μm^2) ± 0.208	JBT	25.581	25.899	25.740
	V	24.378	24.577	24.478
Midpiece length (μm) ± 0.073	JBT	21.610	21.721	21.666
	V	22.613	22.746	22.680

DISCUSSION

It is clear from the analysis that there is no effect of transplantation and consequent development of the males on the sperm dimensions. It is unlikely therefore that the maternal effects that have been found in diallel crosses are mainly due to post-fertilization maternal environment. The other possibilities, not isolable in the present experiment, are maternal cytoplasmic effect and an X chromosome effect. It is possible that some genes located on the X chromosome are found to affect sperm dimensions. Small sampling in this experiment prohibits a serious consideration of these observations, especially, since the variation in head breadth between the two genotypes was so small (the two strains did not differ in their head breadth, though the sperm heads of V strain were shorter

and broader than of the JBT in shape), and as it seems that head breadth is the dimension likely to be influenced by maternal + X chromosome factors (see Chapter V). However, it is certain that non-chromosomal maternal effects are not of any great importance. In view of the results obtained in this experiment and elsewhere (Chapters III and V) in this thesis, it seems worthwhile to attempt isolation of non-chromosomal maternal effects from X chromosome controlled effects in a separate study, using those strains which differ in their head breadth widely and show marked reciprocal effects in F_1s. If sex effects are genetic in origin, it is likely that one would find factors located on X chromosome rather than on the Y chromosome (Chapters III and V), though Y effects have been reported to control the proportion of abnormal spermatozoa in some mouse strains (Krzanowska, 1969; Brozek, 1970).

SUMMARY

Transplantation of blastocysts from one strain to the other and consequent pre- and post-natal development with a different strain as mother has no apparent effect on the spermatozoan dimensions of inbred strains of mice.

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CHAPTER V

DIMENSIONAL CHARACTERISTICS OF MOUSE SPERMATOZOA IN A VARIETY OF GENOTYPES OBTAINED BY CROSSING CBA AND C57 STRAINS AND THEIR BACKCROSSES

Parental sex effects on the spermatozoan dimensions have been observed by various workers (Sharma, 1960; Mori, 1961; Beatty, 1963; Pant, 1972; Chapter II). It is not always easy to interpret them further since they could be attributed to a combination of several factors. Since dimensions are so strongly under additive genetic control, one would expect a priori that some of the genes would be located on one or both of the sex chromosomes and would be responsible for parental sex effects. We have already seen that the Y chromosome does not seem to have a recognizable effect on the sperm dimensions (Chapter III).

The present experiment was planned to tackle this problem with a different approach. Using males with different genotypes, it was possible to produce genetically similar offspring. It was also possible to produce kinds of males that had identical strain-specific autosomal genotypes but contained either the X or the Y chromosome from different strains. In some cases the offspring were genotypically similar, but their male parents had different X chromosomes. The different classes also had different proportions of strain-specific autosomal genes. The various kinds of progeny permitted a study of several factors. These factors were: a Y chromosome effect; an X chromosome effect confounded with a maternal effect; an effect associated with the X chromosome of the male parent; autosomal effects, and dominance effects. This seems to be the first attempt made to isolate so many factors that potentially influence sperm dimensions.

MATERIALS AND METHODS

For the sake of convenience, letters 'A' and 'B' will be used for describing CBA and C57 strain genotypes respectively. The source of the sex chromosomes will be designated by using sub-scripted small letters, 'a' and 'b'. Eight kinds of male parents were produced for the final crossing in this experiment. Table 1 gives the matings that produced the parents, the genotypes of the parents, and the symbols that will be used to describe the genotypes in this Chapter. In this table, the 4thBC(CBA♀ x C57♂)♂ represents a male that was obtained by repeated backcrossing of the F_1 (CBA♀ x C57♂) to CBA♀♀ for four generations. Backcrossing of reciprocal F_1 (C57♀ x CBA♂) to C57♀♀ for four generations produced the 4thBC(C57♀ x CBA♂)♂. The eight kinds of male parents were crossed with CBA and C57 females, giving 16 kinds of offspring. The genotypes of the 16 kinds (cells) are presented in Table 2, and these have been diagrammatically represented in Figure 1. Ten male offspring in each cell, from as many litters as possible, were used for making spermatozoal preparations. The 160 males were killed in a random order. Their ages (calculated as at the midpoint of the 96-hour period during which they were killed) and their body weights (at the time of killing) are presented in Table 3. The method of preparing smears, coding of slides, drawing of spermatozoa and of their measurements were the same as in Chapter II. From each male, duplicate preparations were made, and in each of the 320 slides, 10 spermatozoa were randomly selected and drawn, using one sheet per sperm. Thus, the sampling was : 16 cells, 160 males (10 in each cell), 320 preparations (2 in each male), and 3200 sperms (10 in each preparation). The observations obtained were statistically analysed and converted to μm units. The probability

TABLE 1. GENOTYPES OF MALE MICE OBTAINED BY CROSSING CBA, C57 AND THEIR TWO RECIPROCAL BACKCROSSES WITH THE FEMALES BELONGING TO CBA AND C57 STRAINS OF MICE

Item No.	Matings	Genotype of male offspring				
		Autosomes		sex-chromosomes		Symbols
		CBA (%)	C57 (%)	X	Y	
1	CBA♀ x CBA♂	100.0	0	CBA	CBA	AAX_aY_a
2	C57♀ x C57♂	0	100.0	C57	C57	BBX_bY_b
3	CBA♀ x C57♂	50.0	50.0	CBA	C57	ABX_aY_b
4	C57♀ x CBA♂	50.0	50.0	C57	CBA	ABX_bY_a
5	CBA♀ x 4thBC(CBA♀ x C57♂)♂	98.4375	1.5625	CBA	C57	AAX_aY_b
6	C57♀ x 4thBC(C57♀ x CBA♂)♂	1.5625	98.4375	C57	CBA	BBX_bY_a
7	C57♀ x 4thBC(CBA♀ x C57♂)♂	48.44	51.56	C57	C57	ABX_bY_b
8	CBA♀ x 4thBC(C57♀ x CBA♂)♂	51.56	48.44	CBA	CBA	ABX_aY_a

TABLE 2. GENOTYPES OF THE MALE PROGENY OBTAINED IN THE FINAL GENERATION BY CROSSING CBA AND C57 STRAINS, THEIR CROSSES AND BACKCROSSES

	♀ parents	
♂ parents	AAX_aX_a	BBX_bX_b
AAX_aY_a	AAX_aY_a	ABX_bY_a
BBX_bY_b	ABX_aY_b	BBX_bY_b
ABX_aY_b	0.75A0.25BX_aY_b	0.25A0.75BX_bY_b
ABX_bY_a	0.75A0.25BX_aY_a	0.25A0.75BX_bY_a
AAX_aY_b	AAX_aY_b	ABX_bY_b
BBX_bY_a	ABX_aY_a	BBX_bY_a
ABX_bY_b	0.75A0.25BX_aY_b	0.25A0.75BX_bY_b
ABX_aY_a	0.75A0.25BX_aY_a	0.25A0.75BX_bY_a

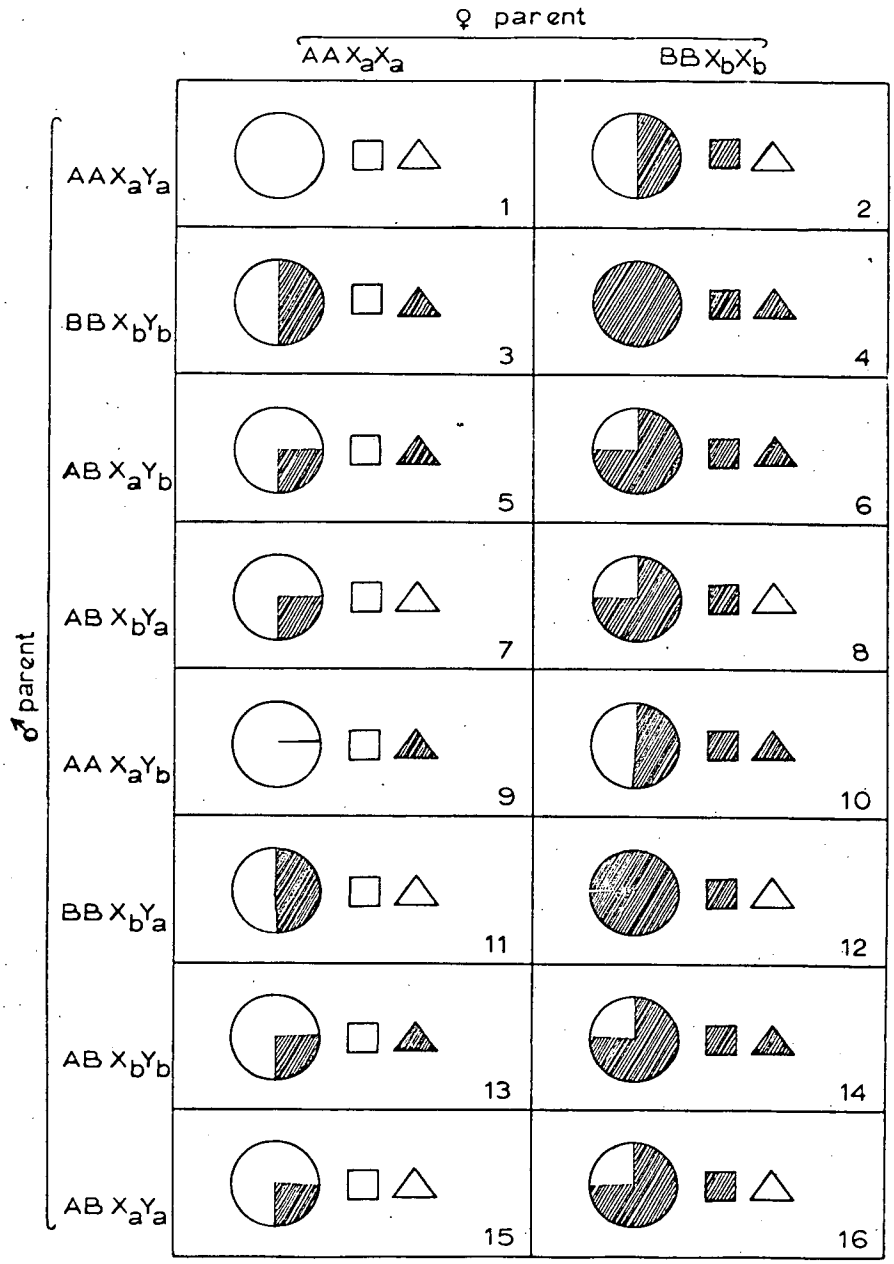

FIGURE 1. Diagrammatic representation of the different genotypes obtained by crossing CBA and C57 strains, their F_1s and backcrosses. Unshaded area represents CBA genotype and shaded area C57 genotype. (Circles - autosomal genes, Squares - X chromosomes, Triangles - Y chromosomes.)

TABLE 3. AGE (DAYS) AND BODY WEIGHT (GMS.), AT THE TIME OF KILLING, OF THE MALE PROGENY OBTAINED BY CROSSING CBA AND C57 STRAINS OF MICE AND THEIR RECIPROCAL CROSSES AND BACKCROSSES.

	♀ parents			
	AAX_aX_a		BBX_bX_b	
♂ parents	Age	Body wt.	Age	Body wt.
AAX_aY_a	106.1 ± 2.5	31.44 ± 1.42	108.0 ± 3.5	36.84 ± 0.91
BBX_bY_b	104.5 ± 0.7	37.25 ± 0.70	104.7 ± 3.5	26.54 ± 0.92
ABX_aY_b	104.1 ± 2.9	33.38 ± 1.16	103.6 ± 2.8	33.13 ± 1.30
ABX_bY_a	104.4 ± 2.8	30.99 ± 0.95	104.0 ± 3.2	34.98 ± 1.28
AAX_aY_b	111.7 ± 0.3	32.08 ± 0.69	104.5 ± 3.0	36.36 ± 1.23
BBX_bY_a	104.4 ± 3.1	33.14 ± 0.49	99.6 ± 3.8	28.12 ± 1.10
ABX_bY_b	103.3 ± 2.7	34.73 ± 1.15	106.6 ± 3.1	34.58 ± 1.11
ABX_aY_a	98.0 ± 2.2	31.47 ± 0.92	101.6 ± 2.7	32.95 ± 0.66

(± standard error)

levels in the tests of significance have been marked as in Chapter II.

RESULTS

The cell means are presented in Table 4. The three dimensions, head area, head breadth and midpiece length, will be termed Y_1, Y_2 and Y_3 respectively. The design of the experiment permitted study of five independent sources of variation that could have influence on the spermatozoan dimensions. These are;

(i) X_1 = a Y chromosome effect. This has been shown to be of no importance in Chapter III.

(ii) X_2 = an X chromosome effect confounded with a maternal effect.

(iii) X_3 = a possible effect of the X chromosome of the male parent.

(iv) X_4 = linear autosomal effect of strain-specific genes.

(v) X_5 = quadratic autosomal (dominance) effect.

Initially, a multiple regression analysis was carried out by the method of Woolf (1951). The following equations were obtained when all the five independent variables were included (numerical values are sometimes given to more decimal places than warranted by the accuracy of the observations. This is merely to avoid "rounding-off error" when such values are used for further calculation);

$$Y_1 = 23.9955 - 0.0175X_1 - 0.1127X_2 + 0.0752X_3 + 2.9277X_4 - 1.2249X_5$$

$$Y_2 = 3.6338 - 0.0056X_1 - 0.0509X_2 + 0.0162X_3 - 0.0178X_4 + 0.1284X_5$$

$$Y_3 = 22.2309 + 0.0140X_1 + 0.0787X_2 + 0.0176X_3 - 0.3341X_4 - 0.2387X_5$$

TABLE 4. MEAN SPERMATOZOAN DIMENSIONS IN THE 16 CELLS (GENOTYPES) PRODUCED BY CROSSING CBA AND C57 STRAINS, THEIR RECIPROCAL F_1S AND THEIR RECIPROCAL BACKCROSSES

		♀ parents					
		AAX_aX_a			BBX_bX_b		
♂ parents	± S.E.	Head area (μm^2) ± 0.183	Head breadth (μm) ± 0.018	Midpiece length (μm) ± 0.045	Head area (μm^2) ± 0.183	Head breadth (μm) ± 0.018	Midpiece length (μm) ± 0.045
AAX_aY_a		24.852	3.746	21.858	24.041	3.590	22.340
BBX_bY_b		24.489	3.672	22.276	22.885	3.577	22.492
ABX_aY_b		24.408	3.702	22.012	23.984	3.608	22.401
ABX_bY_a		24.941	3.753	21.942	23.705	3.603	22.411
AAX_aY_b		24.545	3.697	21.914	23.983	3.566	22.272
BBX_bY_a		23.988	3.614	22.272	22.954	3.580	22.560
ABX_bY_b		24.082	3.651	22.090	23.519	3.575	22.447
ABX_aY_a		24.516	3.659	22.022	23.037	3.548	22.385

Various combinations of the independent factors were explored and revealed that linear effects were important in midpiece length and head area, but not in head breadth. In no case did X_5 contribute significantly. The 16 cell means were then submitted to computer analysis. The X_5 variable was not considered in this analysis. The analysis was done by the automatic elimination facility of the MULTREG programme devised by R.E. Day and R.L. Middleton of the Edinburgh Regional Computing Centre, as presented in the BISRA PEGASUS programme OR/CA/39/65. From the independent variates offered to it the programme selects the best set of variates that give collectively a significant regression, and that individually have significant partial regression coefficients. The remaining variates, whose inclusion would not significantly increase the predictive power of the regression equation, are automatically eliminated[‡]. The results of the analysis are presented in Table 5. In head area, the four variables together removed 79.97 % of the total variation between the 16 cells, and X_4 alone was responsible for as much as 79.01 %. In midpiece length, all the four independent variables removed 94.70 % of the total variation and X_4 accounted for 93.86 %. In head breadth, the total variation removed by the four variables was 76.86 %, and 71.03 % was explained by X_2 alone.

Regressions calculated on these variables alone (X_4 for Y_1 and Y_3, and X_2 for Y_2) gave the following prediction equations;

$$Y_1 = 23.093966 + 1.8030X_4 \quad (\text{where, } X_4 = 0 \equiv C57;\ X_4 = 1 \equiv CBA)$$

$$Y_2 = 3.686912 - 0.1062X_2 \quad (\text{where, } X_2 = 0 \equiv CBA;\ X_2 = 1 \equiv C57)$$

$$Y_3 = 22.575561 - 0.6894X_4 \quad (\text{where, } X_4 = 0 \equiv C57;\ X_4 = 1 \equiv CBA).$$

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‡I am indebted to Mr. Rafiq Shaikh of the Edinburgh Regional Computing Centre for programming and computation of multiple regressions.

The predicted dimensions for the two strains were calculated as follows;

Dimensions	CBA	C57	Source of strain differences
Head area (μm^2)	24.897	23.094	additive variation only (X_4)
Head breadth (μm)	3.687	3.581	X chromosome + maternal effect only (X_2)
Midpiece length (μm)	21.886	22.576	additive variation only (X_4)

As X_4 seemed to be of importance in the determination of head area and midpiece length, the 16 cells were plotted against the X_4 variable in Figure 2. In this figure, the proportion of CBA genotype, which has five classes varying from 0 to 1, is the X_4 variable, and each point of this variable represents a certain combination of CBA and C57 strain specific genes. Each such point can be referred to as an autosomal gene class. A perusal of the figure reveals a clear linear trend in the midpiece length where the variation between cell means within each autosomal gene class is very small. Since the analysis (Table 5) showed that X_2 was the only independent variable affecting the head breadth, it was considered worth while to average those means within each autosomal gene class that belonged to cells born from same strain mother, and thus had the X chromosome of the same strain. In the autosomal gene class 0.5, there were two types of cells, (i) those with an X chromosome from C57 strain, and (ii) those with an X chromosome from CBA strain. In other autosomal gene classes, the cell means within each class did not differ in the strain-specificity of their X chromosomes. Thus, in all autosomal gene classes except 0.5, only

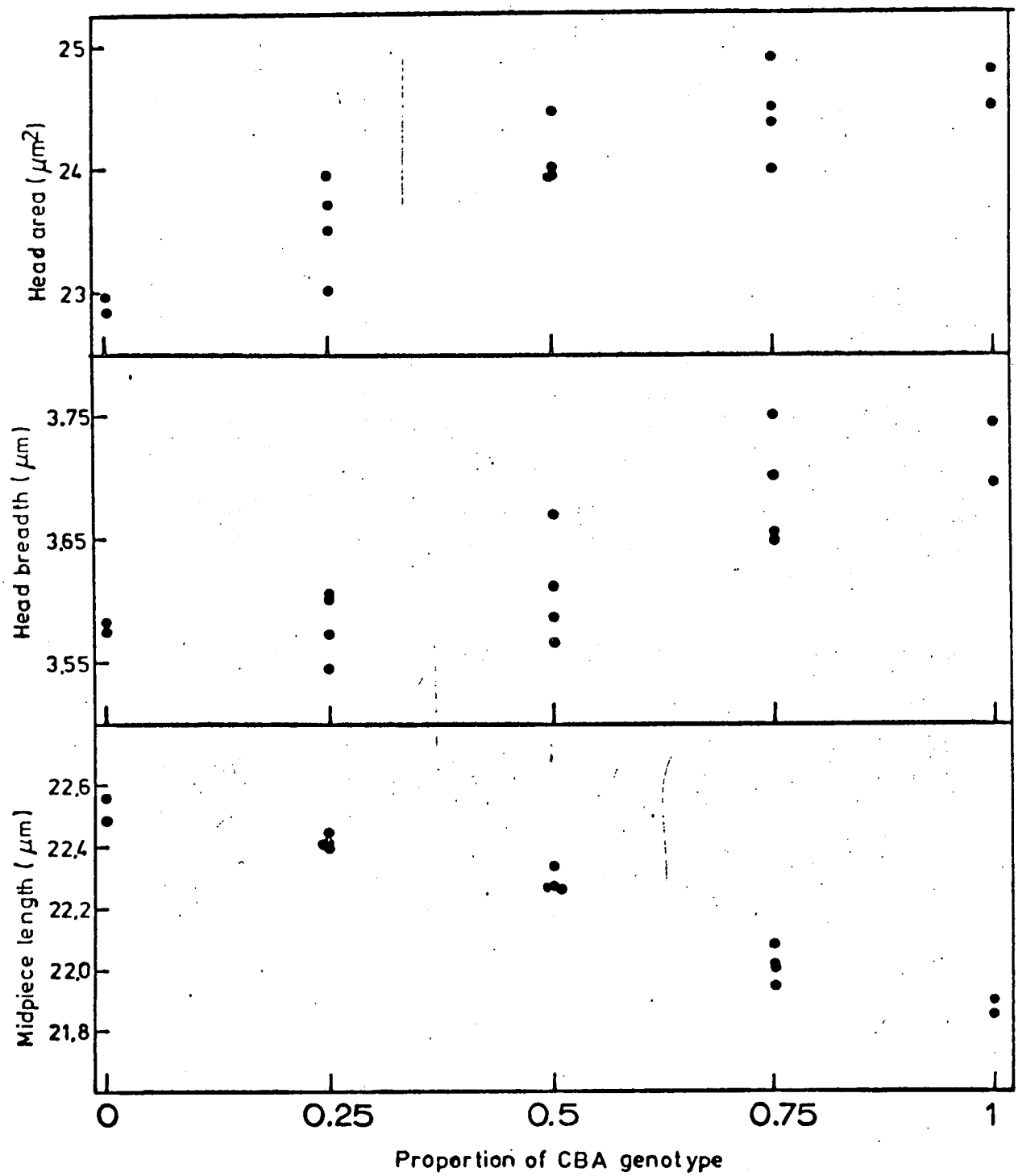

FIGURE 2. The mean spermatozoan dimensions of the 16 genotypic classes (cells) plotted against autosomal gene classes (the X_4 variable). A linear trend in the midpiece length is obvious.

TABLE 5. ANALYSIS OF VARIANCE OF SPERM DIMENSIONS IN CBA AND C57 STRAINS, THEIR CROSSES AND BACKCROSSES

Source of variation	d.f.	Y_1 (head area)		Y_2 (Head breadth)		Y_3 (Midpiece length)	
		SS	MS	SS	MS	SS	MS
Regression on X_1, X_2, X_3, X_4, X_5	5	5.146675	1.029335 ****	0.0511526	0.0102305 ****	0.727285	0.145457 ****
Error	10	1.025130	0.102513 ****	0.0123786	0.0012379 ****	0.032237	0.003224
Regression on X_4	1	4.8760	4.8760 ****	--	--	0.712897	0.712897 ****
Regression on X_2	1	--	--	0.045124	0.045124 ****	--	--
Error	14	1.2958	0.0926 ****	0.018407	0.001315 ****	0.046623	0.003330
Cells	15	6.1718	0.4115 ****	0.063531	0.004235 ****	0.75952	0.050635 ****
Males within cells	144	--	0.023633 ***	--	0.000329 ****	--	0.0019996 ****
Preparations within males	160	--	0.015526 ****	--	0.000157 ***	--	0.0008390
Spermatozoa within preparations	2880	--	0.008343	--	0.000113	--	0.0007074

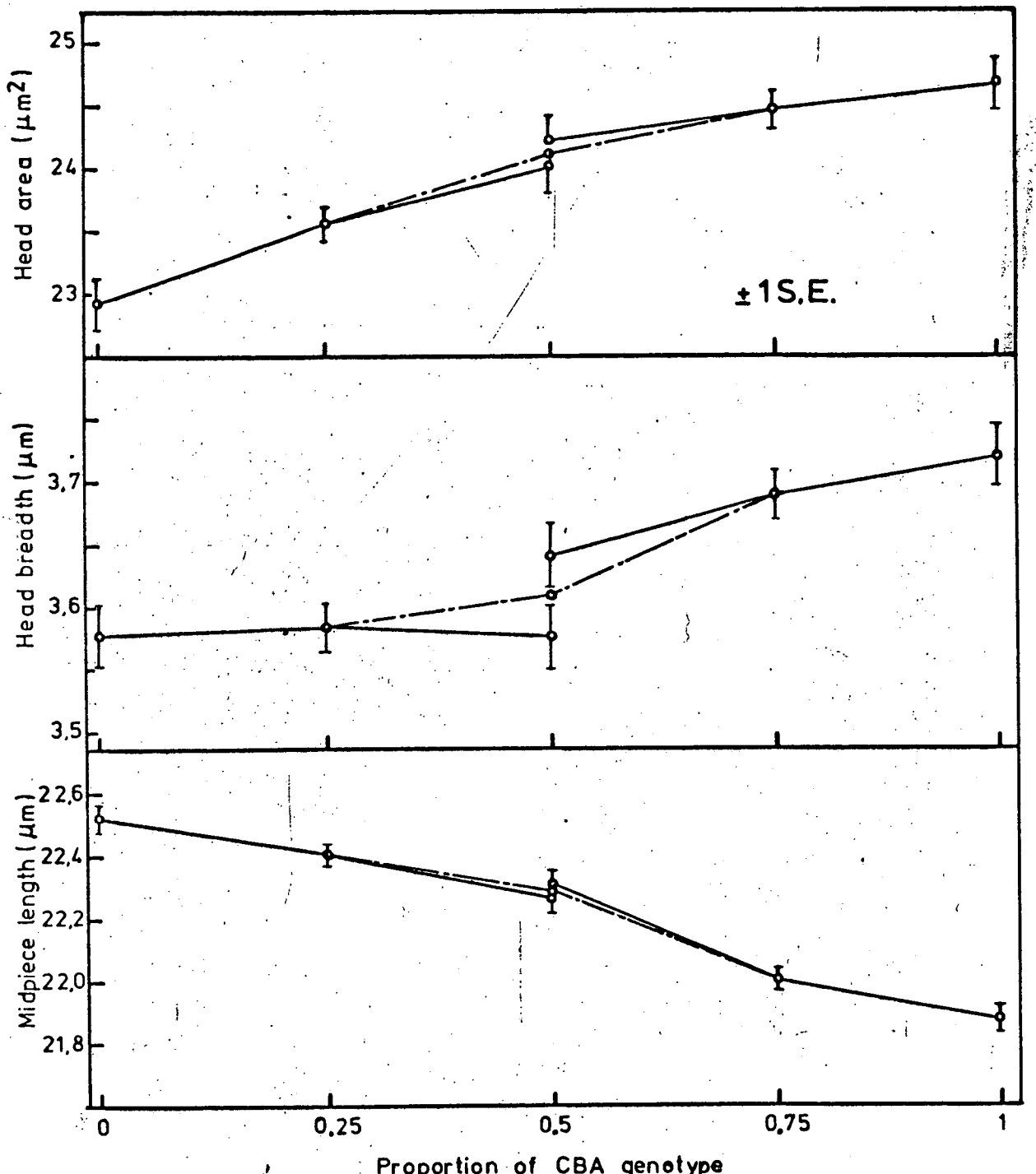

FIGURE 3. The mean spermatozoan dimensions of the 16 genotypic classes (cells) plotted against the autosomal classes. The cell means have been arranged in such a way that each point represents the same autosomal gene proportion and same X chromosome. Such a difference existed only in the 50:50 autosomal class, and there the grand mean irrespective of the X chromosome is connected to other autosomal class means by broken lines.

one average was obtained, and there were two averages in the 0.5 class. These are diagrammatically represented in Figure 3. Head area and midpiece length in this figure show a clear linear trend. In head area, however, there is slight evidence of curvilinearity, possibly suggesting dominance. It is interesting to note, in this figure, that the head breadth shows a different trend from other dimensions. The head breadth of CBA strain was larger than of the C57 strain. If additive effects were important, one would have expected a gradual increase in the head breadth as the proportion of CBA genes increased. In the figure, however, the change in head breadth from autosomal gene class 0 (no CBA genes) to 0.5 (50 % CBA genes) was very small; and in fact was zero if only those autosomal gene classes are considered that contain an X chromosome of C57 strain (i.e. were born from C57 mothers). This particular observation, with the results on the other dimensions, will be discussed later in this chapter.

Within cells, the analysis was the same as in previous chapters, i.e. males within cells, preparations within males, and spermatozoa within preparations. The variation between males within cells was used to test the significance of residual error in the analysis of variance of regressions. This residual error was found to be highly significant in Y_1 and Y_2, but not in Y_3. This showed that in Y_3 only additive effects were important and there was nothing else left to be explained. In Y_1 and Y_2, however, there could be factors, other than those considered in this experiment, which have remained unexplained. Except for complicated types of dominance effects (which we did not study), there were no other factors that we could have visualized.

DISCUSSION

In all earlier studies and in the preceding chapters of this thesis, we have seen that the spermatozoan morphology seems to be under strong genetic control. Evidence relating to the influence of paternal sex on the sperm phenotype (Beatty, 1963, 1969; Krzanowska, 1969, 1972; Brozek, 1970) led us to explore the possibility of a non-autosomal genetic mechanism that may affect sperm dimensions. Such non-autosomal factors are likely to be found on the sex chromosomes, though there exist other possible mechanisms (see discussion in Beatty, 1969). The sex chromosomes of the mouse are known to carry genes, e.g. the coat colour gene on the X (Fraser, Sobey & Spicer, 1953; Lyon, 1960), the histocompatibility gene on the Y (Eichwald & Silmser, 1955). Mittwoch (1967), discussing the possible modes of action of sex chromosomes, states, "The orthodox mode of action of chromosomes is via genes which they carry. There is of course not the slightest doubt that sex-chromosomes carry genes.... There is every indication the X-chromosome bears its full complement of genes, although there is usually a striking scarcity of genes on the Y chromosome."

In the type of the experiment that is being presented, it was assumed that the two sex chromosomes do not cross-over and tend to remain intact, and Ohno (1969) is of the opinion that the X chromosome remains unaltered maintaining the original X of the common ancestor in most of the placental mammals, including the mouse.

The role of the sex chromosomes in spermatogenesis is well understood in Drosophila (Meyer, 1972), and the influence of the Y chromosome on fertility and abnormality of spermatozoa has been shown in mice by Krzanowska (1969, 1972) and Brozek (1970).

Two dimensions of spermatozoa, the head area and the midpiece length, have been found to be controlled by linear (additive) effects. This is especially marked in the case of midpiece length where linear effects seem to be the only factors and variation within each autosomal class is very small. These observations, along with Woolley's (1970) findings, support the argument made in Chapter III relating to midpiece length. The two head characteristics had earlier (in Chapter III) shown a nearly linear trend with successive generations of backcrossing, although there the backcrossing effect was confounded with the maternal effect. The head breadth has been found to be influenced by factors related to the strain of the mother (maternal + X chromosome effect). It may be for this reason that Illisson (1969) observed a significant maternal effect in her study on the shape of the sperm head. If maternal effects are important, it would be interesting to isolate an X chromosome effect from a cytoplasmic or environmental maternal effect. Observations made in Chapter IV indicate that non-chromosomal maternal effects may not be important determiners of sperm dimensions, and thus, these observations point to a possible X chromosome effect. But, unfortunately the results obtained in that Chapter were inconclusive for head breadth as the variation between the two strains was so small. It seems desirable at this stage that a study be conducted on head breadth in relation to maternal effect in various ways, including perhaps the effect of transfer of fertilized eggs between strains that vary greatly in their head breadth.

The head breadth behaves differently from the other dimensions in several respects. In the first experiment (Chapter II), in the diallel analysis, the mean square due to the female parent effect was larger than that due to the male parent effect. Both these effects were tested against a common error term and their respective significance levels were also different

($P < 0.005$ in the former, and $P < 0.05$ in the latter). Though, statistically the excess of the female parent effect over the male parent effect is not significant, it seems that the female parents may be contributing more to the additive effects, and it might be possible to reveal this if a larger number of strains were used, increasing the degrees the freedom. It is also interesting to note that in F_1, the head breadth shows negative heterosis, though the magnitude of heterosis does not differ significantly from zero. The results obtained in this thesis may be compared with the results of other workers (Table 6). From Sharma's (1960) results, from calculations made from Beatty's

TABLE 6. A COMPARISON OF THE CROSSBRED AND PUREBRED MEAN DIFFERENCES IN THE SPERM DIMENSIONS OBTAINED IN THIS THESIS WITH THE RESULTS OF OTHER STUDIES

	(Crossbred mean) minus (Purebred mean)			
			This thesis	
Dimensions	Sharma (1960)	Beatty (1969)	Chapter II	This Chapter
Head breadth (μm)	- 0.01	- 0.049	- 0.024	- 0.062
Head area (μm^2)	+ 0.70	+ 0.13	+ 0.454	+ 0.793
Midpiece length (μm)	+ 0.20	+ 0.115	+ 0.124	+ 0.267

(1969) data and from the results obtained in this thesis, the mean head breadth of crosses is shown to be regularly slightly lower than that of the purebreds.

A perusal of head breadth in Figure 3 shows that there might be a negative curvilinearity in the trend suggesting dominance of the genotype controlling the smaller head breadth of C57 strain. Since, in the

computer analysis, only the X chromosome effect was significant, the validity of such an assumption is not without doubt.

In head area, as in midpiece length, factors other than those concerned with the additive effects does not seem to be of any importance.

Thus, different dimensions of spermatozoa behave differently in their patterns of inheritance. Bahr & Zeitler (1964) have shown that different dimensional characteristics of spermatozoa vary quite independently of one another, and Beatty (1970), in a review, also reaches similar conclusions.

The present study shows that the Y chromosome has no influence on the measurements made on the dimensions of spermatozoa. Krzanowska (1969) has also reported that, despite the fact that the Y chromosome affects the proportion of morphologically abnormal spermatozoa, it did not seem to have any influence on the shape of the sperm head. In Drosophila, Meyer (1972) has shown that the Y chromosome affects the size of spermatozoa, the number of Y chromosomes in an individual's karyotype being directly related to the length of their spermatozoa. However, some recent work in our laboratory (Upadhyaya, 1971) shows that the Y chromosome alone may not directly affect the length of spermatozoa in Drosophila, and it may be necessary to consider other factors.

SUMMARY

Three dimensions of spermatozoa, head area, head breadth and midpiece length, were studied in a variety of genotypically different groups of mice produced by crossing and backcrossing of CBA and C57 strains.

The 16 kinds of progeny differed either in the proportion of strain-specific autosomal genes, or in one or the other of the sex chromosomes. In some cases, genotypically similar progeny were obtained by crossing genotypically different parents. Upon statistical analysis, the variation between the 16 genotypic classes was attributable to additive gene effects in head area and midpiece length, whereas variation in the head breadth was attributable to the identity of the maternal strain (and therefore, possibly to the X chromosome). Thus, the dimensions appeared to be under the influence of different factors to varying degrees. The Y chromosome had no effect on the variation between the 16 genotypic classes.

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APPENDIX
OF PUBLICATIONS

PROC. INT. SYMP. THE GENETICS OF THE SPERMATOZOON
Edinburgh, Aug. 16–20, 1971

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pp. 116–120

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PATTERNS OF INHERITANCE IN THE MIDPIECE LENGTH OF MOUSE SPERMATOZOA

By

K. P. Pant

Marked genetic control of dimensional characters of spermatozoa of various species is well known. On the basis of very high heritability estimates for the midpiece length of mouse spermatozoa (*Woolley & Beatty*, 1967), it was possible to increase or decrease the midpiece length by selecting males on the basis of their spermatozoan phenotype (*Woolley*, 1971). To study the patterns of inheritance in this characteristic of spermatozoa, a 4 × 4 diallel cross was made between four inbred strains of mice in the present work. The strains were V (*Beatty*, 1954), JBT (JBT/Jd), CBA (CBA/Fa) and C57 (C57 BL/Fa).

The age of the parents chosen for crossing ranged between 44 and 64 days at the time the matings were set up. Males from first litters of the 16 possible crosses (henceforth called cells) were used for the experiment. Five males were randomly selected in each cell; they came from 2 to 4 different matings. Litter identity within cells was ignored as a source of variation (*Beatty & Sharma*, 1960). The eighty males, which varied between 58 and 63 days in age, were killed in a random order by stunning and subsequent neck dislocation. Immediately after killing, duplicate nigrosin-eosin preparations of the contents of the vasa deferentia were made from each mouse by the method of *Beatty & Sharma* (1960). All the preparations were made within a period of 2 days in a controlled environment room. Each of the 160 coded slides was optically projected at a linear magnification of x 6130, and the lengths of 20 midpieces

of randomly selected spermatozoa were drawn along their principal axis on separate sheets of paper. The lines were measured with a rotameter.

The whole experiment was repeated a second time, using second litters. The procedure was the same except for the optical magnification which was x 6139, and the age of the males varied between 94 and 137 days. The means over both experiments are shown in Table 1.

The measurements were analysed statistically using *Hayman's* (1954) model of diallel analysis. The variance on 15 degrees of freedom between the 16 cells was partitioned as shown in Table 2. Within each cell, the variance was partitioned in a hierarchical order as follows: males within cells, preparations within males, and spermatozoa within preparations. The variance for males within cells was tested against preparations within males, and the latter against spermatozoa within preparations. All other sources were tested against males within cells. All interactions between first and second experiments were found to be non-significant, and the two sets were, therefore, pooled. The final analysis is presented in Table 2.

Within cells, the relative contribution of each source of variation was: males within cells, 5.5 %; duplicate preparations within males, 2.9 %; spermatozoa within preparations, 91.6 %. The block interaction (B) on one degree of freedom represents the overall difference between the two experiments. This may be due to the difference in the ages of the males in the two experiments, or more likely to a small magnification factor error. The difference between grand means of the two experiments was about 0.9 % of the combined grand mean. The interactions between sources of variation in the diallel and the two experiments were all non-significant. *Beatty & Mukherjee* (1963) also did not find any effect of the age of mice on the midpiece length of spermatozoa. It

Table 1.
Mean spermatozoan midpiece length (in microns) in the 16 cells of a diallel cross between 4 inbred strains of mice.

		♀ parent				♂ parent mean
		V	JBT	CBA	C57	
♂ parent	V	22.590	22.341	22.325	22.779	22.509
	JBT	22.005	21.626	21.756	22.288	21.919
	CBA	22.316	21.822	21.793	22.318	22.062
	C57	22.792	22.386	22.201	22.605	22.496
♀ parent mean		22.426	22.044	22.019	22.498	22.247

Table 2.
Analysis of variance of midpiece length from the diallel cross of 4 inbred strains of mice.

	Source of variation	d.f.	Mean squares
either	♂: male parents	3	0.72655**
	♀: female parents	3	0.50179**
or	a: parental effects averaged over sex	3	1.19586**
	c: parental sex effects	3	0.03247**
	d: maternal effects due to variation within individual sets of reciprocal crosses	3	0.01440*
	b: dominance effects	6	0.02739**
	b_1: overall dominance effect	1	0.09205**
	b_2: dominance of individual lines	3	0.00675
	b_3: dominance of individual crosses	2	0.02602**
	t: total of diallel	15	0.25950
	B: block interaction between two sets of diallel	1	0.31330**
	Bt: pooled block interactions	15	0.00285
	M(C): males within cells	128	0.00410**
	P(M): preparations within males	160	0.00166**
	S(P): spermatozoa within preparations	6080	0.00102

Significance levels: ** $P<0.005$
* $0.005<P<0.01$

is reasonable, therefore, to assume that age had no effect on the variations in the midpiece length. The midpiece length did not show any evidence of specific dominance of individual lines (b_2). All other sources of variation were highly significant. It seems that a larger portion of the variance between cells is due to additive effects (a) of genes, and that there are marked male (♂) and female (♀) parent effects. *Beatty* (1963) reported much significant additive variation with a tendency for greater male than female parent effects in a diallel analysis of sperm dimensions. Midpiece length is known to be a highly

heritable characteristic, reflected here by the large additive genetic portion in the variation between cells. Consistent large male parent effects have been observed by *Beatty* (1969), and *Krzanowska* (1969) has suggested a possible influence of the Y chromosome on the morphology of spermatozoa. It should therefore be possible to confirm the Y effect in studies on spermatozoa from males whose Y chromosome has been replaced with that of another strain by repeated backcrossing. Such a possibility is currently under investigation. In view of the findings of *Woolley* (1970, 1971), it seems unlikely that the amount of mitochondria in the body cells of the parents will influence the length of the midpiece in the spermatozoa of their progeny, which otherwise as a paternal cytoplasmic effect could have been a possible reason for a male parent effect. *Szollosi* (1965) has also reported that spermatozoan mitochondria soon degenerate in the eggs after fertilization.

It is difficult to analyse the female parent effects in further detail. These could be X chromosome linked effects, but unlike with the Y chromosome, there is crossing over between the two X chromosomes in the hybrid and this makes the individual X chromosome lose its strain identity. Pre- and postnatal maternal effects could be investigated to some extent by intra-uterine transfer of fertilized eggs from one strain to the other.

A highly significant parental sex effect (c) indicates that the effects of the two sexes are not the same and strains have different effects when used as male and as female parents.

Crossing experiments can also be used to see if there is a difference in the within-male variances of sperm dimensions of crossbred and inbred males, as evidence of a haploid effect. If the haploid genome of a gamete influences its phenotype, one would expect increased variance in the sperm dimensions of crossbred males. In the present experiment, however, the pooled within-male variance of crossbred animals (0.200) was actually slightly *lower* than in purebreds (0.215), and hence there is no evidence of a haploid effect.

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going to effect overall economy of a cow because length of lactation period can be controlled by force drying the animals at a desired stage of lactation.

The phenotypic relationships of age at first calving with other economic traits are of low magnitude and non-significant.

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Studies on the physical characteristics of buffalo spermatozoa: A note

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The object of the work was to study if the interferometrically determined physical quantitative characteristics, i.e. refractive index, thickness, concentration of solid matter per 100 ml of water and dry mass per unit area of the midpiece and the anterior part of the head of buffalo spermatozoa varied between different breeds kept at different farms.

From each of the 4 breeds, 2 bulls were randomly chosen. From each bull 2 semen samples were collected on different days in winter. From each

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sample, a smalldrop was taken and added to 5 ml of buffered formol saline (pH 7.0; Mukherjee, 1963) and left at the room temperature for half an hour. Two preparations were made by pouring the sperm suspension on 2 clean, grease-free slides and dried at 37°C. Before examination, the slides were washed in running tap-water for about 40 to 50 minutes and dried. One of the slides was mounted in normal saline and the other in a protein solution prepared by dissolving 1 g of bovine plasma albumin (Fraction V, L. Light) to 10 ml of saline (Barer and Joseph, 1955). Final concentration of protein in the solution was determined from the equation mentioned below. In the equation, values of μ_1 and μ_2 were determined by means of an Abbe refractometer (Bausch and Lomb).

$$\text{Concentration of protein (Cp)} = \frac{\mu_1 - \mu_2}{\alpha}$$

where α is the specific refractive increment, which was taken as 0.0018 (Barer, 1956), considering spermatozoa as cells rich in protein and protein-bound substances.

In a slide, 6 to 7 spermatozoa, apparently normal in morphology and without a protoplasmic droplet at the midpiece, were selected at random. On each spermatozoon, phase measurement was made at the anterior part of the head and at the midpiece by means of a Baker interference microscope with 40×shearing objective and a half shade 10×eye-piece. The light source was a Beck Tenslite lamp with arrangement for Köhler illumination. A monochromatic green filter, which allowed light of 5,460 A° wavelength to pass, was used. From the measurements on the spermatozoa, the average phase change at the two regions of spermatozoa was determined for a slide. The average was used to determine the physical characteristics; and for the

Table 1. Means of the spermatozoan characteristics of buffalo bulls of four breeds

Breeds	Animal No.	Head				Midpiece			
		Refractive index	Thickness (μ)	Solid matter (g/100 ml water)	Dry mass ($g \times 10^{-13}/\mu^2$)	Refractive index	Thickness (μ)	Solid matter (g × 100 ml water)	Dry mass ($g \times 10^{-13}/\mu^2$)
Murrah	1	1·413	1·600	44·225	7·065	1·413	1·751	44·355	7·536
	2	1·402	1·868	38·630	7·203	1·408	1·818	41·687	7·434
	Mean	1·408	1·734	41·428	7·134	1·410	1·784	43·021	7·485
Nili	1	1·408	1·686	41·796	6·982	1·425	1·467	50·820	7·412
	2	1·413	1·545	44·032	6·807	1·423	1·422	49·706	7·064
	Mean	1·410	1·615	42·914	6·894	1·424	1·445	50·263	7·238
Jaffarabadi	1	1·407	1·613	40·845	7·051	1·406	1·707	40·450	6·733
	2	1·404	1·663	39·272	6·537	1·413	1·608	44·325	7·123
	Mean	1·405	1·638	40·058	6·794	1·409	1·657	42·387	6·928
Surti	1	1·396	1·905	34·775	6·630	1·394	2·169	33·499	7·232
	2	1·482	1·596	37·837	6·115	1·400	2·098	36·602	7·677
	Mean	1·399	1·751	36·306	6·372	1·397	2·133	35·050	7·454
Overall means		1·405	1·684	40·176	6·799	1·410	1·755	42·680	7·276

Table 2. Analysis of variance of spermatozon characterics

Source of variation	d.f.	Mean squares							
		Head				Midpiece			
		Refractive index	Thick-ness	Solid matter	Dry mass	Refractive index	Thick-ness	Solid matter	Dry mass
Between breeds	3	0·0000962	0·0150	31·960	0·406	0·0005127*	0·333*	154·545*	0·323
Between animals within breeds	4	0·0000459	0·0471	12·228	0·146	0·0000272	0·005	7·832	0·138
Error	8	0·0000681	0·0325	20·330	0·537	0·000112	0·0482	34·477	0·181

* $P<0·005$.

Table 3. Physical characteristics of spermatozoan midpiece of buffalo bulls of four breeds arranged in ascending order

Characteristics	Value of critical difference	Breed means			
Refractive index	0·004	Surti (1·397)	Jaffarabadi (1·409)	Murrah (1·410)	Nili (1·424)
Thickness (μ)	0·052	Nili (1·445)	Jaffarabadi (1·657)	Murrah (1·784)	Surti (2·133)
Concentration (g/100 ml matter)	1·965	Surti (35·050)	Jaffarabadi (42·387)	Murrah (43·021)	Nili (50·263)

purpose, the formulae described by Mukherjee (1966) were used. Accordingly:

$$\text{Refractive index } (\mu) = \frac{\varnothing_1}{\varnothing_1 - \varnothing_2}(\mu_2 - \mu_1) + \mu_1$$

where $\varnothing_1$ and $\varnothing_2$ are the average phase changes at a particular region of spermatozoon, mounted in normal saline (of refractive index μ_1), and protein solution (of refractive index μ_2) respectively.

$$\text{Thickness } (t) = \frac{\varnothing_1 \times 5460}{360\ (\mu - \mu_1)} \times 10^{-4}$$

$$\text{Concentration of solid matter in g per 100 ml of water } (C) = \frac{\varnothing_1}{\varnothing_1 - \varnothing_2} C_p$$

$$\text{Dry mass in g per } \mu^2 (D) = C_t \times 10^{-13}$$

Means of the spermatozoan characteristics of buffalo bulls of the 4 breeds are given in Table 1. Differences observed between animals within a breed and between breeds were studied in the analysis of variance (Table 2). In the analysis, error mean squares (on 8 degrees of freedom) was used to test the mean squares between animals within a breed, and the latter mean squares (on 4 degrees of freedom) was used to test the mean squares between breeds. The means of the 3 characteristics of the midpiece are arranged in ascending order in Table 3. The figures under the same bar did not vary significantly at 5 per cent level of probability.

The physical characteristics of the anterior part of the head did not vary between breeds or between animals within a breed. It is doubtful if the physical characteristics of the sperm heads were as identical as the results of the present experiment suggest. The μ, t and C, but not the D, of midpiece appeared to vary

between breeds. The variations between bulls were not significant and were very small.

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A note on comparative studies on the fore-stomach wall of buffalo (*Bubalus bubalis*) and ox (*Bos indicus*)

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An experiment was undertaken to make comparative studies on the fore-stomach wall of buffalo and ox. Tissue pieces of the fore-stomachs from 10 bullocks and 10 buffaloes were collected from the middle of the parietal surface of rumen, reticulum and omasum within ½ to 1 hour from the time of slaughter. They were fixed in 10 per cent buffered formalin and 6-μ-thick sections cut. The sections were stained with haematoxylin and eosin, and Mallory's aniline blue-collagen stain (Anon., 1960).

The wall of the fore-stomachs in ox and buffalo consisted of 4 distinct tunics, viz. (i) the tunica mucosa, (ii) submucosa, (iii) muscularis, and (iv) serosa. The values on the thickness of different tunics of fore-stomachs are given in Fig. 1 for rumen, reticulum and omasum respectively.

Tunica mucosa

The mucosa was lined by stratified, squamous, cornified epithelium. The core of the ruminal papillae, reticular ribs and omasal laminae was formed by the lamina propria and submucosa. These two connective tissue layers were inseparable in rumen and reticulum, but were distinctly separable in omasum by a layer of longitudinally oriented smooth muscle fibres, the muscularis mucosae, which also ascended to the core of omasal laminae below the lamina propria mucosae. In buffalo the core of reticular ribs had a thick band of smooth muscle fibres, extending throughout the height of the reticular rib. The fibres of this muscle layer were oriented along the length of the rib and are said to be the continuation of muscularis mucosae of oesophagus (Trautmann and Fiebiger, 1957). In the ox this band was confined below the tip of reticular rib. The core of omasal laminae presented connective tissue with blood vessels and smooth muscle layers. The two lateral muscle layers were the continuation of muscularis mucosae of the wall of omasum and the third central layer was the extension of the inner circular muscle layer of the tunica muscularis of the wall

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THE EFFECT OF SEASONS ON THE SPERM DIMENSIONS OF BUFFALO BULLS

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Summary. Dimensional characteristics and proportions of live spermatozoa in permanent nigrosin–eosin preparations from the semen samples of Murrah buffalo bulls were studied in different seasons. Between bulls, all the characteristics varied significantly. Between seasons, all the characteristics, except length of head and mid-piece, varied significantly. Environmental temperature and humidity both appeared to influence the dimensional and enumeration characteristics of the spermatozoa. The percentage of live spermatozoa decreased with increase in air temperature and humidity.

There is ample evidence that the dimensional characteristics of the spermatozoa of various species are under genetic control; a review on the subject has been published recently (Beatty, 1970). In tropical bulls, the seasons have been shown to influence the sperm dimensions (Mukherjee & Singh, 1966; Mishra, Singh & Roychoudhury, 1969), and the reproductive performance of buffaloes is known to be more influenced by the seasons than that of other cattle. In the present study, buffalo spermatozoa were studied in four seasons for the following characteristics: head length, breadth, area and shape (head length/head breadth), mid-piece length, breadth and area and the proportion of unstained spermatozoa.

The semen samples from four Murrah buffalo bulls were collected at an interval of 3 days during the peaks of the seasons (December to January—Winter; February to March—Spring; May to June—Summer; August—Autumn). Sixteen samples, four in each season from each bull, were examined. From each sample, three permanent nigrosin–eosin (Hancock, 1951) preparations were made on clean grease-free slides. The 192 slides were coded and the code was not available until all the observations had been made on the spermatozoa. In each slide, four unstained and morphologically normal spermatozoa were randomly selected and camera lucida drawings of the head and the midpiece of these spermatozoa were made on separate sheets of paper at a linear magnification of ×5975. It has already been shown (Beatty & Sharma, 1960) that mean differences smaller than the limits of optical resolution can be validly studied. From the measurements on four spermatozoa, made by the method of Rajwar & Mukherjee (1970), the averages of each characteristic were calculated

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TABLE 1

ANALYSIS OF VARIANCE OF THE SPERM CHARACTERISTICS OF MURRAH BUFFALO BULLS IN DIFFERENT SEASONS

Sperm characteristic	*Bulls* (*d.f.* = 3)		*Seasons* (*d.f.* = 3)		*Collections within bulls* (*d.f.* = 12)		*Bulls × Seasons* (*d.f.* = 9)		*Seasons × Collections within bulls* (*d.f.* = 36)		*Error* (*d.f.* = 128)	
	MSS	*%TV*	*MSS*	*%TV*	*MSS*	*%TV*	*MSS*	*%TV*	*MSS*	*%TV*	*MSS*	*%TV*
Head length	1·653***	65·2	0·028	0·1	0·017	0·0	0·020	0·6	0·013	4·0	0·016	30·1
Head breadth	0·182***	19·9	0·289***	32·0	0·013	2·1	0·010	0·7	0·009	1·8	0·008	43·5
Head area	29·879***	52·7	4·463***	6·7	0·510	0·0	0·482	0·8	0·647*	7·9	0·371	31·9
Head shape	0·066***	31·9	0·022***	9·8	0·003	1·6	0·003	0·5	0·003	2·5	0·002	53·7
Mid-piece length	6·332***	59·3	0·141	0·0	0·119	1·6	0·162*	3·3	0·077	0·0	0·078	35·8
Mid-piece breadth	0·055***	4·2	0·036***	3·5	0·005	0·7	0·002	0·0	0·002	0·0	0·029	91·6
Mid-piece area	0·766**	5·5	2·746***	22·2	0·293	2·6	0·137	0·0	0·219*	11·3	0·139	58·4
Percentage of unstained spermatozoa†	289·904	5·4	1405·059***	39·1	34·274	0·0	121·467***	14·5	64·755***	29·0	7·867	12·0

MSS = Mean sum of squares, %TV = Percentage of total variance.
Significance levels: no superscript, $P > 0{\cdot}05$; * $P = 0{\cdot}05$ to $0{\cdot}025$; ** $P = 0{\cdot}025$ to $0{\cdot}01$; *** $P < 0{\cdot}005$.
† Transformed to angles (arcsin).

TABLE 2

MEANS OF SPERM CHARACTERISTICS OF MURRAH BUFFALO BULLS AND THE RANGE OF VARIATION IN AIR TEMPERATURE AND RELATIVE HUMIDITY IN DIFFERENT SEASONS

Sperm characteristic and seasonal temp. and humidity	*Winter*	*Spring*	*Summer*	*Autumn*
Head length (μm)	7·63	7·66	7·63	7·69
Head breadth (μm)	4·71	4·77	4·75	4·89
Head shape (head length/head breadth)	1·63	1·61	1·61	1·57
Head area (μm^2)	29·79	30·11	29·99	30·95
Mid-piece length (μm)	11·57	11·48	11·41	11·55
Mid-piece breadth (μm)	0·66	0·71	0·66	0·62
Mid-piece area (μm^2)	7·70	8·16	7·61	7·19
Percentage of unstained spermatozoa	92·2	88·3	80·1	78·0
Average and range of				
Maximum temp. (°C)	24·41(22·0 to 27·0)	31·57(24·2 to 35·5)	42·39(38·0 to 45·5)	32·91(29·0 to 37·0)
Minimum temp. (°C)	4·37(1·0 to 9·5)	11·35(6·2 to 18·0)	26·02(20·0 to 32·0)	25·27(23·0 to 27·0)
Daytime temp. (°C)	15·63(13·3 to 18·0)	24·42(20·2 to 28·5)	38·17(34·2 to 41·1)	29·16(25·6 to 32·3)
Daytime relative humidity (%)	58·93(43·0 to 83·3)	38·17(28·6 to 52·6)	24·50(15·0 to 42·6)	81·98(70·3 to 93·6)

for each slide. These averages were then studied by analysis of variance and the results were finally converted to μm and μm^2. The percentage of unstained spermatozoa was scored by counting 100 randomly selected spermatozoa in each slide and analysed after angular transformation. In the analysis, source of variation was partitioned as shown in Table 1. Mean squares (MS) between bulls was tested against the Bulls × Seasons interaction. To test the MS between collections within bulls, the interaction MS between seasons and collections within bulls was used. To test the MS between seasons, no single interaction was found to be a valid error term. Composite MS for seasons as well as for error and their respective degrees of freedom were therefore computed according to the method of Johnson & Keeping (1952).

Analysis of variance and percentage variance components are given in Table 1. All the mensuration characteristics of spermatozoa varied significantly between bulls. The percentage of unstained spermatozoa, however, showed no significant variation between bulls. The bulls used in the present experiment were selected on the basis of semen quality tests (including unstained sperm percentage), and this may be responsible for the reduced variation between bulls. All the sperm characteristics, except length of head and length of mid-piece, varied between seasons. A comparison of variance components shows that the variation between seasons was greater than between bulls in the head breadth, mid-piece area and the proportion of unstained spermatozoa. Mid-piece breadth was equally influenced by the seasons and the bulls. The head area varied more between bulls than between seasons.

The means of the sperm characteristics in different seasons are given in Table 2. The head breadth and head area were minimal during winter and maximal during autumn when the air temperature was almost as high as in summer and was associated with very high relative humidity. As a result of the change in the head breadth, head shape was minimal during autumn and maximal during winter. Between spring and winter, the characteristics of the head did not vary. It appears, therefore, that with the increase in atmospheric temperature, the sperm head tended to become broader and this change was more marked in autumn when the relative humidity ranged between 70% and 94%. Thus, relative humidity seems to influence the sperm dimensions of buffaloes. Mukherjee & Singh (1966) reported no effect of relative humidity on the sperm dimensions of Hariana bulls.

The mid-piece breadth and area were minimal in autumn and maximal in spring. Between summer and winter, these characteristics did not vary. From all the studies that have been made on various animals, it is now clear that the mid-piece length is relatively free of environmental influence. Its heritability in mouse has been shown to vary between 76% and 97% (Woolley & Beatty, 1967; Woolley, 1970).

A decrease in the proportion of unstained spermatozoa with an increase in air temperature and humidity is expected in view of several reports including those of Waites & Setchell (1964), and Moule & Waites (1963). However, this decrease is much greater than that reported by Mukherjee & Singh (1966) for the proportions of unstained spermatozoa at different seasons in Hariana bulls.

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THE MELANIZING ACTIVITY OF THE SEMEN OF BUFFALO BULLS AND ITS RELATION TO THE PROPORTION OF LIVE SPERMATOZOA

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Melanizing activity of rabbit semen was reported by Beatty (1956). The activity —colour reaction when semen is supplied with dihydroxyphenylalanine— varied between males with different pelt colours: black rabbits showed intense melanizing activity and albinos none. It was further observed that the pale colour of semen was a characteristic of dark pelted bucks, and that the colour of the semen was, in some way, related to its melanizing activity. Mukherjee (1964) studied the melanizing activity of the semen of bulls, goats and rams, and observed that the activity varied between animals and between ejaculates from the same animal. Although there was no appreciable relationship between the coat colour of these animals and the melanizing activity of the semen, the yellow semen samples showed stronger melanizing activity than the non-yellow ones. The proportion of 'live' (unstained in eosin–nigrosin) spermatozoa was uniformly less in strongly melanizing semen samples than in weakly melanizing ones. In the present experiment, an attempt was made to study the melanizing activity of buffalo semen and its relationship to the proportion of 'live' spermatozoa.

For the experiment, twenty-two buffalow bulls of Murrah breed were available. All the bulls were jet black in colour, and were in regular use for artificial insemination. In all, fifty-four semen samples (four from each of two bulls, three from each of thirteen bulls, and one from each of the remaining seven bulls) were collected by means of an artificial vagina and tested for melanizing activity. The procedure for testing melanizing activity was same as that described by Mukherjee (1964) for bulls, and the colour reaction was graded according to the scale described by Beatty (1956).

Only two bulls (Nos. 530 and 372) were found to be positive for melanizing activity. The semen samples from these two bulls and from four other bulls (Nos. 399, 73, 471 and 405), which showed no melanizing activity and were randomly selected, were used to study the effect of various levels of cold shock on the proportion of 'live' spermatozoa. For this purpose, four semen samples were collected from each of the six bulls on different days (a total of twenty-four semen samples). From each sample, immediately after collection, a part was withdrawn and tested for melanizing activity. The remaining part was

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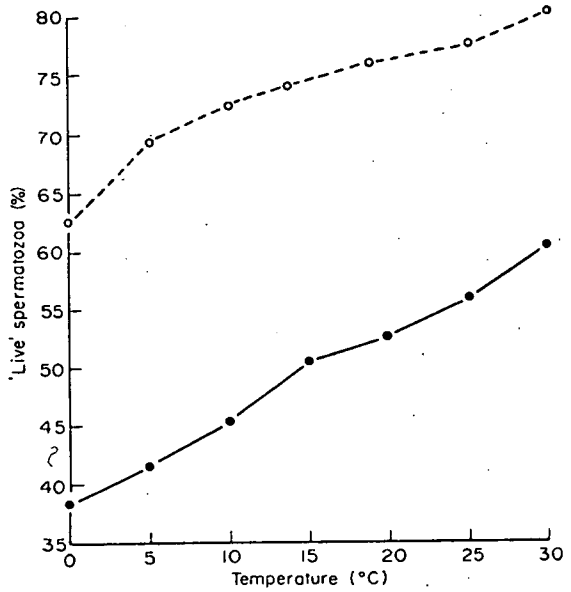
held in a water bath at 33±1° C. The effect of various levels of cold shock was studied according to the procedure described by Mukherjee (1964).

In both the groups (melanizing and non-melanizing) of semen samples, the mean percentage of 'live' spermatozoa decreased with increasing difference in the temperature of semen and the stain. The mean percentage was less in the



TEXT-FIG. 1. Percentage 'live' spermatozoa of buffalo bulls at various levels of temperature shock. ○, Non-melanizing; ●, melanizing.

TABLE 1

ANALYSIS OF VARIANCE OF 'LIVE' BUFFALO SPERMATOZOA

Source of variation	*Degrees of freedom*	*Mean squares*
Groups	1	21640·2****
Bulls within groups	4	8175·5****
Semen samples within bulls	18	275·7****
Treatments	6	1041·6****
Groups × treatments	6	22·9*
Bulls × treatments	24	34·6****
Error	109	10·0

**** $P<0.005$. * $0.025<P<0.05$.

melanizing than in the non-melanizing group at all levels of temperature shock (Text-fig. 1). The difference observed in the percentage of live spermatozoa was subjected to analysis of variance (Table 1). The mean square for groups (tested against the mean square for samples within groups) was highly significant. As the interactions were significant, the composite mean square for treatment and for error and their respective degrees of freedom were calculated

(Johnson & Keeping, 1952), and tested for significance. The variation due to treatments was highly significant.

The intensity of colour reaction varied between gradings of + and + +. In bulls, goats and rams also, the colour reaction varied between similar gradings (Mukherjee, 1964) though in black or nearly black rabbits, it may be as high as + + + + (Beatty, 1956). As the buffalo bulls used in the present experiment were of uniform black colour and the melanizing activity in their semen was weaker than that reported in dark pelted rabbits (Beatty, 1956), it appears that there is no relationship between the melanizing activity and the coat colour of buffalo bulls. Of the two bulls showing melanizing activity, the semen of one (No. 530) was yellow in colour.

The trend of variations in the percentage of 'live' spermatozoa after various levels of temperature shock appeared linear in the two groups and not curvilinear as reported by Mukherjee (1964) in bulls, goats and rams.

The percentage of 'live' spermatozoa is directly related to the metabolic activity of semen and indirectly to the fertility and resistance to cold shock of spermatozoa (Bishop & Hancock, 1955). The melanizing activity of the semen, therefore, may be worth considering when buffalo bulls are selected for artificial insemination, or for a study of the metabolic activity and the resistance to cold shock of the spermatozoa.

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Effect of preservation in carbon dioxide and oxygen diluents on the characteristics of buffalo spermatozoa

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ABSTRACT

The experiment was conducted to study the effect of preservation of buffalo semen in oxygenated (gassed with oxygen), deoxygenated (gassed with carbon dioxide) and control (ungassed) diluents on the morphology and livability of spermatozoa. In all, 12 collections, 4 from each of the 3 bulls of Murrah breed, were obtained. Samples were diluted and stored at 5°C for 24 and 72 hours. Permanent nigrosin-eosin preparations were made before and after dilution. Dimensional measurements of optically projected spermatozoa were made, and proportion of live (unstained) sperms was scored. Dimensional characteristics were not affected by preservation in either oxygenated or deoxygenated diluents. Midpiece area, however, appeared to have decreased in the control diluent after 72 hours of preservation. Proportion of live spermatozoa decreased after 72 hours of preservation in all the diluents.

In a series of experiments (Mukherjee and Singh, 1967, 1968a, b; Kumar and Mukherjee, 1968) it was observed that increase or decrease of oxygen in the inspired air influenced the morphology of mouse and cock spermatozoa; such changes could also be produced by chemical anoxia (caused by atropine and adrenaline) of the visceral organs including testes and *vas deferens* (Mukherjee and Ayyagari, 1969). The object of the present experiment was to study *in vitro* effect of oxygenated and deoxygenated diluents on the cytomorphology of buffalo spermatozoa.

MATERIAL AND METHODS

Three Murrah buffalo bulls, being used for semen collection at the artificial Insemination Centre attached to the Indian Veterinary Research Institute, Izatnagar, were available. Twelve collections were made, 4 from each bull, using an artificial vagina. From each semen sample, immediately after collection, 1 drop was withdrawn and mixed with 3 drops of nigrosin-eosin stain maintained at 32°C. After 2 minutes, 3 smears were made on clean grease-free slides. The slides were dried on a warm stage at 37°C and mounted in D.P.X.

For dilution and preservation of semen, Illini Variable Temperature diluent (Van-Demark and Sharma, 1957) with modifications was used. The buffer solution of the dilutor had sodium citrate dihydrate, 20·01 g; sodium bicarbonate, 2·01 g; potassium chloride, 0·40g; glucose, 3·00 g; sulphanilamide, 3·00 g; and distilled water, 1,000 ml (Sharma, 1960). The constituents of buffer solution were dissolved in distilled water by boiling. The solution was cooled to room temperature. This was then divided into 3 portions. To one portion carbon dioxide gas was passed (by bubbling) for 15 minutes (CO_2 diluent). To another portion oxygen was passed for 15–20 minutes (OXG diluent), and the third portion served as control (CNT diluent). To each of the portions, 10 per cent egg-yolk was added and mixed. To each of the egg-yolk-mixed diluents, semen from the same sample was mixed in the proportion of 1 part of semen to 3 parts of the diluent. The diluted semen samples were put in air-tight ampoules and stored

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in a refrigerator at 5°C. After 24 and 72 hours of preservation, permanent nigrosin-eosin smears were made as before dilution. There were altogether 252 slides. These were coded to veil their identity before examination.

Projected images (final magnification × 6,170) of 4 unstained spermatozoa, selected at random, were drawn on separate sheets of paper (only head and midpiece were drawn; head and midpiece in this paper refer to those of spermatozoa). For projecting images of spermatozoa a projector was used. It consisted of a horizontally mounted microscope with an oil-immersion (97 ×) and an ocular (15 ×) objective. The source of illumination was a high-intensity mercury vapour lamp with control unit on adjustable stand. The whole system was similar to the one used by Beatty and Napier (1960a). Measurements on the drawings of spermatozoa were made according to the methods described by Beatty (1961), Beatty and Napier (1960a, b) and Pant and Mukherjee (1971). Proportion of unstained spermatozoa was scored out of 100 sperms counted in each slide. Probability levels in the tests of significance are marked as follows: * P=0·050–0·025; ** P=0·025–0·010; *** P=0·010–0·005; **** P<0·005.

RESULTS AND DISCUSSION

The main results are presented in Table 1. Differences in the characteristics of spermatozoa after 24 and 72 hours of preservation were studied separately by analysis of variance and the results are presented in Table 2. In the analyses, treatment interaction mean squares were used for testing the main effects of treatments. For all characteristics of spermatozoa, except proportion of unstained spermatozoa at 24 and 72 hours and the characteristics of the midpiece at 24 hours, the interaction mean squares were not significant and were, therefore, pooled. For other characteristics, composite mean squares for treatments and for error were calculated (Johnson and Keeping, 1952). Mean squares between bulls were tested against mean squares between collections within bulls, and mean squares between collections within bulls were tested against interaction mean squares between treatments and collections within bulls. The interaction mean squares were tested against residual (error) mean squares.

All the characteristics of spermatozoa, except midpiece breadth, varied between bulls. After 24 hours of preservation none of the characteristics varied between treatments. After 72 hours of preservation the

Table 1. Mean spermatozoan characteristics of buffalo bulls after 24 and 72 hours of preservation

Spermatozoan characteristics	0 hour (fresh)	24 hours				72 hours			
		CNT	CO_2	OXG	Mean	CNT	CO_2	OXG	Mean
Head length (μ)	7·5000	7·5254	7·4507	7·5222	7·4995	7·5079	7·4739	7·4909	7·4909
Head breadth (μ)	4·7194	4·7484	4·7214	4·7126	4·7275	4·7159	4·7496	4·7201	4·7285
Head area (μ²)	29·0615	29·1666	28·8855	29·2034	29·0957	28·9748	29·0589	28·8619	28·9643
Head shape	1·5955	1·5840	1·5803	1·5982	1·5875	1·5923	1·5735	1·5875	1·5844
Midpiece length (μ)	11·5823	11·5886	11·5463	11·6463	11·5938	11·4612	11·5998	11·4503	11·5038
Midpiece breadth (μ)	0·6681	0·6498	0·6616	0·6667	0·6594	0·6551	0·6715	0·6694	0·6653
Midpiece area (μ²)	7·7409	7·5278	7·6211	7·7309	7·6266	7·5112	7·7940	7·6789	7·6616
Unstained spermatozoa (%)	84·53	77·91	78·14	80·31	78·79	75·72	75·22	76·44	75·79

Table 2. Analyses of variance of spermatozoan characteristics of buffalo bulls after 24 and 72 hours (figures in italics) of preservation

Source of variation	d.f.	Head length	Head breadth	Head area	Head shape	Midpiece length	Midpiece breadth	Midpiece area	Unstained spermatozoa (%)*
Treatments	3	0·04270	0·008932	0·72981	0·002719	0·06168	0·002485	0·36580	188·814
		0·00725	*0·008748*	*0·31493*	*0·003400*	*0·22199*	*0·001988*	*0·53876*	*385·223*****
Bulls	2	5·40263****	0·259404****	142·13068****	0·110703**	12·11255****	0·004684	7·98759***	4935·072****
		4·26189****	*0·264554*****	*120·85710*****	*0·094730***	*12·11851*****	*0·005385*	*9·77208****	*5679·318*****
Collections within bulls	9	0·04269****	0·019940	2·36591****	0·017370****	0·52729	0·005971	1·07946	257·736****
		0·03341	*0·197290**	*2·58856****	*0·016880*****	*0·36438*	*0·005333****	*1·17767*****	*224·655*****
Treatments × bulls	6	0·04469	0·007566	0·74410	0·001811	0·56024***	0·001342	0·53006***	64·255****
		0·02950	*0·007776*	*1·36216*	*0·001520*	*0·08275*	*0·000672*	*0·08592*	*31·157***
Treatment × collections within bulls	27	0·01091	0·010009	0·57037	0·002347	0·27270	0·003581**	0·61151****	31·717
		0·03357	*0·008485*	*0·97103*	*0·001820*	*0·22961*	*0·001513*	*0·24522*	*33·290*
Pooled interactions	33	0·01705	0·009563	0·60199	0·002249	—	—	—	—
		0·03283	*0·008354*	*1·04212*	*0·001760*	*0·20307*	*0·001360*	*0·21623*	—
Residual (error)	96	0·02068	0·018259	1·15241	0·001754	0·19916	0·001424	0·22086	17·045
		0·02335	*0·010534*	*1·03280*	*0·001150*	*0·15079*	*0·001455*	*0·25751*	*13·920*

*Transformed to angles (arcs in) before analysis.

characteristics, except percentage of unstained spermatozoa, did not vary between treatments. The midpiece area, however, approached the 5 per cent level of significance, and a critical test showed that the midpiece area in CNT diluent, after 72 hours of preservation, was significantly less than in freshly collected semen. The percentage of unstained spermatozoa declined after 72 hours of preservation, and the decline was uniform in the 3 diluents.

Mukherjee and Dott (1960) observed no significant variation in the length and breadth of bull spermatozoan head preserved up to 72 hours in egg-yolk-citrate diluent, but observed a decrease in these characteristics of spermatozoa when preserved in egg-yolk-glycine diluent. Tomar *et al.* (1964) reported that the head length of buffalo spermatozoa tended to decrease with preservation. In the present study, the mensuration characteristics of spermatozoan head of buffalo bulls were not influenced by preservation in CNT, CO_2 and OXG diluents up to 72 hours.

The length and the breadth of the midpiece were not influenced by preservation. Midpiece area, however, decreased in CNT diluent after 72 hours. As the size of the midpiece is correlated with its enzymatic activity (Ayyagari and Mukherjee, 1970), it appears that the metabolic activity of midpiece decreased in CNT diluent. The midpiece area did not vary either in CO_2 or OXG diluents. It is possible that the enzymatic activity of the midpiece remained relatively unaltered in the two diluents. It may be mentioned that carbon dioxide gas in diluents may cause anabiosis (Milovanov and Khabibulin, 1933), and immotility of human (Shettles, 1940) and bull (Sharma, 1960) spermatozoa.

The percentage of unstained spermatozoa decreased with preservation after 72 hours. As the percentage of unstained spermatozoa is related to fertility (Bishop and Hancock, 1955), it appears that the fertility of buffalo semen decreased after 72 hours of preservation . Mukherjee and Dott (1960) observed that preservation of bull semen in egg-yolk-citrate or egg-yolk-glycine diluents did not influence the proportion of live spermatozoa. Normal fertility of bull semen in CO_2 diluents up to 7–8 days were reported by Van-Demark and Sharma (1957), Scott and Hardenbrook (1958) and Hayden *et al.* (1960). Dunn and Foote (1958), however, could not find favourable results with extenders as compared to egg-yolk-citrate diluents.

On the *in vitro* effect of oxygen, available reports suggest that the preservation of semen of ram (Honmode, 1967) and cock (Proudfoot, 1966; Proudfoot and Stewart, 1967) in an atmosphere of oxygen improves the fertility of spermatozoa. Normal fertility of fowl semen stored in CO_2 diluents was reported by Harris and Hobbs (1964). Proudfoot (1966), however, could not confirm the results and reported a decrease in the fertility of fowl semen preserved in deoxygenated diluents.

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ABSTRACT OF THESIS

Name of Candidate KANTA PRASAD PANT

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Title of Thesis Patterns of Inheritance in the Dimensions of Mouse Spermatozoa.

Three dimensional characteristics of spermatozoa were studied for their modes of genetic behaviour. The material consisted, at the start, of four highly inbred strains of mice (V, JBT, CBA and C57), and the dimensions were : Head breadth (HB), Head area (HA) and Midpiece length (ML).

After a diallel crossing of these strains, it was observed that the variation between different genotypes was largely due to strong inter-strain differences (used as a measure of additivity of the genes causing strain-specificity). In an alternative statistical analysis, this strain effect was reflected as strong male and female parent effects. There was a significant parental sex effect in ML, meaning that the strains when used as the male parents had different effects than when used as female parents. Mean dominance was observed in HA and ML, and in ML the variation between individual sets of crosses in their degree of dominance was also marked. Other sources of variation were of minor importance. Most of the variation within each genotypic group was attributable to the variation between spermatozoa within preparations (biologically, between spermatozoa within a male). The variations between males within genotypes and between preparations within males were considerably smaller. The within-male variance was not different in crossbred and inbred progeny, indicating an absence of haploid effects (expression of the haploid genome contained in each normal spermatozoon) on spermatozoan dimensions. The data bearing on this important question of haploid effect are probably the most extensive so far available. The HA appeared to be correlated with the body size. CBA and C57 strains appeared to vary most.

These two strains (CBA and C57) were chosen and the Y chromosome of one strain was put on to the genetic background of the other strain by repeated backcrossing of each reciprocal F_1 male to the female belonging to its mother's strain through several generations. A comparison of backcrosses after six generations of backcrossing (upgrading) to pure strains showed that there was no evidence of a Y chromosome effect. This observation was further supported by the findings obtained in another study where a number of genotypes were produced by crossing the two strains and their F_1s and backcrosses in different ways. The latter experiment, the first of its kind, showed that only additive effects were important in the determination of HA and ML, but HB was likely to be controlled by a maternal effect that was confounded with an X chromosome effect.

In an attempt to isolate the non-chromosomal maternal effect from an X chromosome effect, fertilized eggs were reciprocally transferred from one female to another of a different strain (foster-mother) in the blastula stage. The observations on the resulting progeny showed that the non-chromosomal maternal effects were likely to be of very small magnitude, if they existed.

Thus, the results obtained in this investigation show a very marked genetic control of the dimensional phenotype of spermatozoa. But the different dimensional phenotypes do not follow a single pattern of genetic behaviour common to them all. The idea that the strain specificity of the dimensions is solely due to additivity of the genes controlling them, has been put to test, and it has been shown that the HB behaves differently from other dimensions. A possible control of the HB by the X chromosome has been demonstrated, and a further exploration of this character has therefore become necessary. For the first time, it has been possible to exclude the Y chromosome completely as a potential source of variation in the quantitative studies on these dimensions of spermatozoa.

Use other side if necessary.