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Factor responsible for spermatozoan abnormality located on the *Y* chromosome in mice

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1. INTRODUCTION

During the studies on heterosis it was found that the fertilization rate in one of our inbred strains of mice, KE, is rather low. In fertile matings usually more than 20% of ova remain unfertilized, many of them containing spermatozoa within the perivitelline space (Krzanowska, 1960). Also the time elapsing between the copulation and penetration of the vitellus by spermatozoa is longer in this strain (Krzanowska, 1964). This physiological inefficiency is accompanied by a high proportion of sperms with morphologically deformed heads. In the KE strain the percentage of such abnormal sperms amounts to 17% (Krzanowska, 1962). This is a primary abnormality, developing during spermiogenesis (Godowicz, 1965).

To inquire into the mode of inheritance model of these characters, crosses between the KE and KP strains were made. It appeared (Krzanowska, 1966) that fertilization rate as well as the proportion of abnormal sperms show a remarkable heterosis effect in the F_1 generation and that the reciprocal F_2 crosses differ significantly with respect to both characters. Several types of backcrosses were performed to see whether sex linkage or cytoplasmic effects were involved in this difference. It became obvious that the *Y* chromosome was the factor responsible. In all cases a higher proportion of abnormal spermatozoa was found in the type of backcross where the *Y* chromosome was derived from the KE strain.

In preliminary experiments similar results were obtained in crosses between the KE and CBA strains. It seemed worthwhile to investigate this problem further, as in spite of the importance of the *Y* chromosome as the male sex-determining factor in mammals (Welshons & Russell, 1959), our knowledge about the genes located on it is very scarce. Till now only the locus causing the rejection of male skin transplants by the female is known to be *Y*-linked in mice, although it is not strain specific (Billingham & Silvers, 1960).

The aim of the present experiments was to introduce the *Y* chromosome from strain CBA, where male fertility is high, to the genetical background of the strain KE and to see whether this would improve sperm quality and fertilization rate. Positive results would indicate that a factor affecting male fertility is really located on the *Y* chromsome and can be separated from the other polygenes influencing the same character.

2. MATERIAL AND METHODS

Two inbred strains of mice were used: 41–47th generation of KE (genotype ccaabb—Krzanowska, 1965) and CBA (genotype CCAABB—introduced from Edinburgh to Cracow in 1956 and bred in our Department since 1964). The reciprocal F_1 crosses were made and then two types of backcross were performed by mating KE females with F_1 (KE × CBA) males or with F_1 (CBA × KE) males (designated B_1 and $B_{1''}$ respectively). The strain of the female is always denoted first. From this first type of cross six generations of backcrosses were obtained according to the following scheme:

$$B_1 = \text{KE} \times F_1 \text{ (KE} \times \text{CBA)},$$
$$B_2 = \text{KE} \times B_1,$$
$$B_3 = \text{KE} \times B_2, \text{ etc.}$$

Only brown agouti (CcAabb) males were used to sire litters with the aim to develop a coloured strain congenic with KE. For that reason the degree of genetical agreement with the tester strain, which after six generations is expected to attain 98% (Falconer, 1960), has probably not been reached yet.

From the fifth generation two types of the backcrosses were made differing as to the source of the Y chromosome: one, designated B_6 by mating eight KE females with six B_5 males and the reciprocal designated $B_{6''}$ by mating seven B_5 females with three KE males. No attention was paid to the colour of the mice this time.

A similar experiment was repeated in the sixth generation using KE and B_6 mice. Nine KE females were mated with five B_6 males to obtain the B_7 generation while 10 B_6 females mated with six KE males gave birth to $B_{7''}$.

The majority of the B_7 and $B_{7''}$ males were tested for fertility by mating them with their sisters or KE females as well as with highly fertile F_1 (KE × Outbred) females. On the next day after copulation plug was found the females were killed, their Fallopian tubes flushed with 0·9% NaCl and the proportion of fertilized ova estimated under the microscope. Unfertilized eggs were mounted between the slide and the coverslip and fixed to examine the chromatin structure (Krzanowska, 1960).

From the beginning of the experiment all males were examined for the percentage of abnormal spermatozoa. After killing the male by cervical dislocation the content of the vas deferens was squeezed to one drop of 0·9% NaCl solution and mixed by pipetting. Smears were prepared, air dried and fixed for 10 min in absolute alcohol:ether (1:1) mixture. After drying the smear was moistened with a drop of water, covered with a coverslip and observed under the phase-contrast microscope with a green filter. Four hundred spermatozoa from each male were counted and the proportion of specimens with abnormal heads was estimated. A coding system was used so that the genotype of the male was not known at the time of scoring. For statistical treatment all percentages were transformed to angles (Snedecor, 1955).

Five KE and five CBA males were submitted to karyotype analysis. Cells were taken from the bone marrow of the femur without pretreatment with colcemide. Air-dried preparations were made and stained with aceto-orcein.

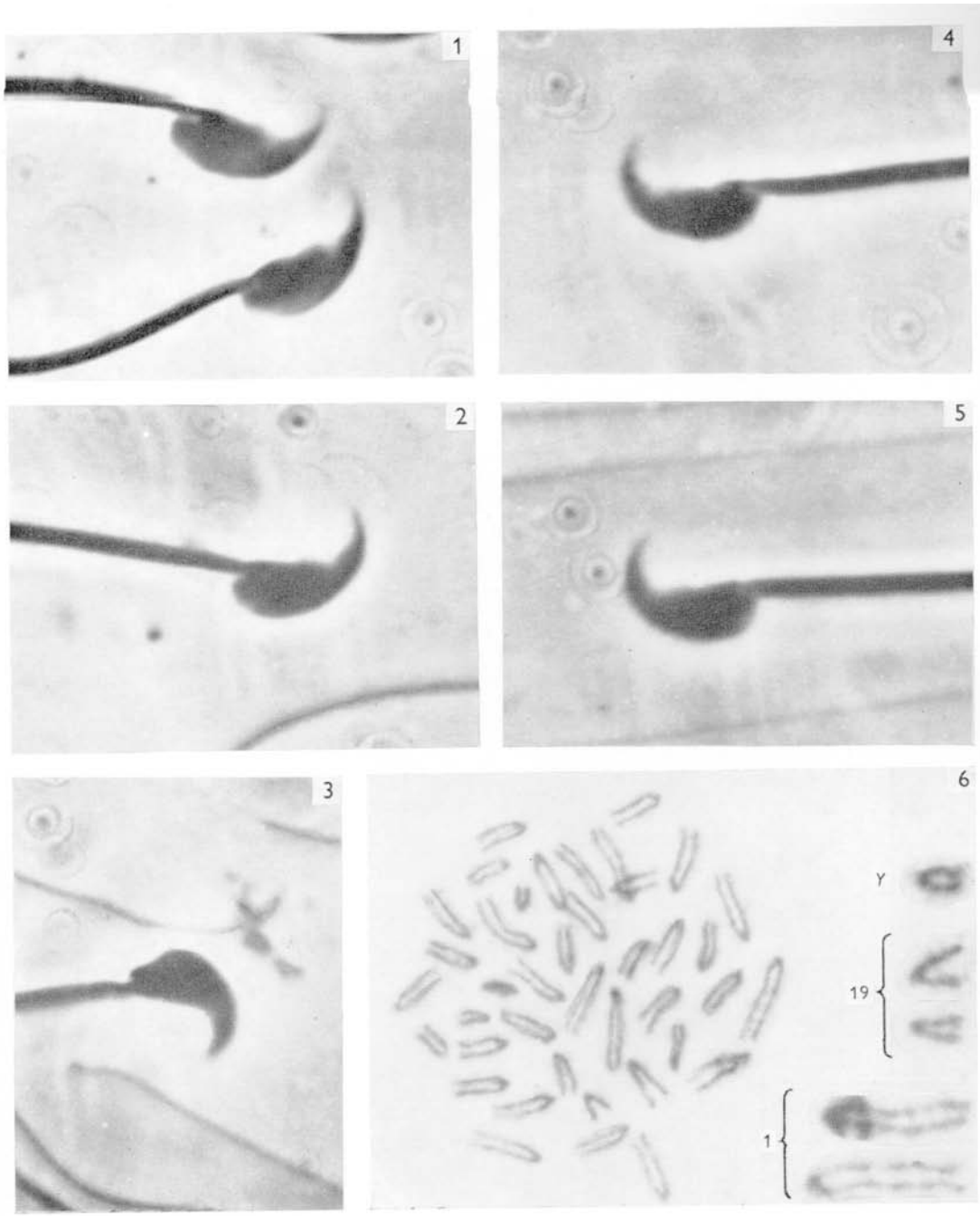

Fig. 1. Two normal spermatozoa of CBA strain.

Fig. 2. Normal spermatozoon of KE strain.

Fig. 3. Abnormal spermatozoon of KE strain.

Fig. 4. Normal spermatozoon of $B_{7''}(B_6 \times \mathrm{KE})$ generation.

Fig. 5. Normal spermatozoon of $B_{7'}$ $(\mathrm{KE} \times B_6)$ generation.

Fig. 6. Chromosomal plate of KE male (chromosome pairs 1, 19 and chromosome *Y* shown separately under high magnification).

Figs. 1–5. Preparations fixed in alcohol–ether, unstained, phase-contrast; fig. 6 fixed in acetic alcohol, stained in acetic orceine.

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3. RESULTS

(i) *Percentage of abnormal spermatozoa*

The two inbred strains differ considerably with respect to the proportion of abnormal spermatozoa (Plate 1, fig. 3) which amounts to 5·9 % in CBA and as much as 16·1 % in the KE strain (Table 1). The frequency distributions of males possessing a given percentage of abnormal spermatozoa practically do not overlap (Fig. 1).

Table 1. *Mean proportion of abnormal spermatozoa in the inbred strains KE and CBA, their crosses and backcrosses*

Males	Source of Y chromosome	No. of males	Mean proportion of abnormal spermatozoa	
			%	Angle ± S.D.
Inbred CBA	CBA	17	5·9	14·0 ± 2·56 **
Inbred KE	KE	27	16·1	23·6 ± 3·14
F_1 (KE × CBA)	CBA	6	1·9	7·9 ± 1·96 **
$F_{1''}$ (CBA × KE)	KE	5	4·6	12·3 ± 0·77
B_1 (KE × F_1)	CBA	23	5·4	13·4 ± 2·67 **
$B_{1''}$ (KE × $F_{1''}$)	KE	19	11·7	20·0 ± 3·81
B_2 (KE × B_1)	CBA	2	10·5	—
B_3 (KE × B_2)	CBA	6	8·9	—
B_4 (KE × B_3)	CBA	8	9·4	—
B_5 (KE × B_4)	CBA	5	9·4	—
B_6 (KE × B_5)	CBA	19	9·2	17·6 ± 2·58 **
$B_{6''}$ (B_5 × KE)	KE	17	19·4	26·1 ± 3·70
B_7 (KE × B_6)	CBA	30	10·2	18·6 ± 2·28 **
$B_{7''}$ (B_6 × KE)	KE	25	16·1	23·6 ± 2·88

** The difference between the reciprocal crosses significant at 1 % level.

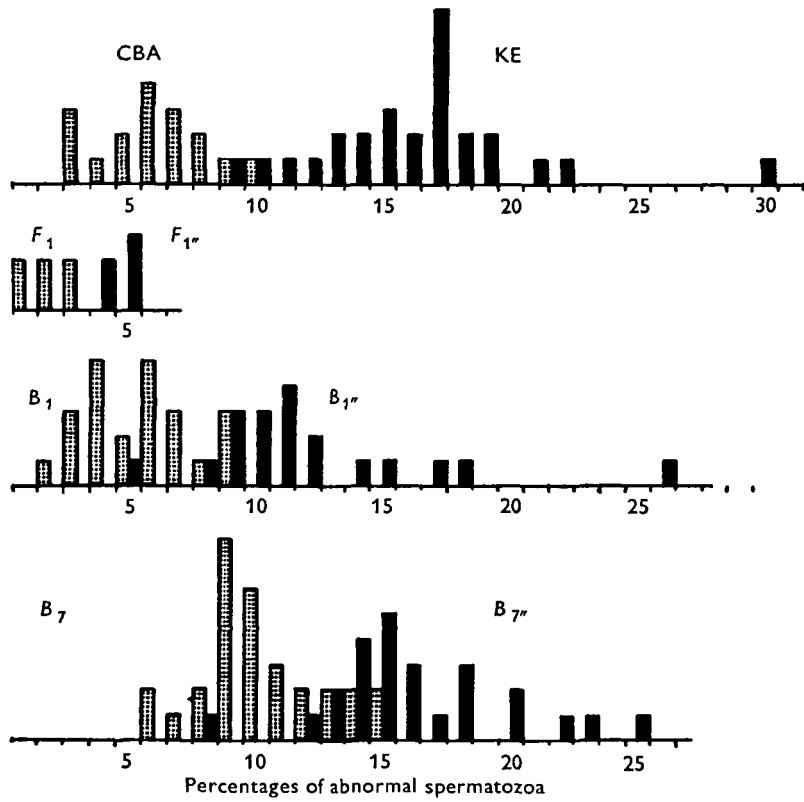
In the F_1 generation a heterosis effect was observed and the difference between the reciprocal crosses was significant in spite of the scarcity of material examined. The variance of the F_1 crosses was very low.

In the backcrosses an obvious segregation of genotypes has taken place, especially when $F_{1''}$ (CBA × KE) males were used to mate with KE females ($B_{1''}$). In that case the variance was very high (Table 1) and at least five males with the percentage of abnormal spermatozoa as high as in the KE strain were recovered, while they were not found in the reciprocal backcross B_1 (Text-fig. 1). The reciprocal difference was highly significant.

Only a few males were bred in the next generations of the backcrosses and for that reason statistical treatment of these data was abandoned. The mean percentage of abnormal spermatozoa, which amounted to 5·4 % in the B_1 generation, rose to 10·5 in B_2 and then did not change significantly till B_7.

The reciprocal backcrosses developed in the sixth and in the seventh generation gave consistent results. In both cases where the Y chromosome was of KE origin

($B_{6''}$ and $B_{7''}$ respectively) these crosses showed a significantly higher proportion of abnormal spermatozoa, similar to that of the KE strain (Table 1, Text-fig. 1). That is just what one would expect, since both sex chromosomes (X and Y), as well as most autosomes, were derived from the KE strain. In the reciprocal backcrosses (B_6 and B_7) where the genetical background was the same but the Ychromosome of CBA origin, the percentage of abnormal heads was much lower,



Text-fig. 1. Frequency distributions of KE, CBA and backcross males possessing a given percentage of abnormal spermatozoa.

although significantly higher than in the CBA strain. This indicates that the two inbred strains differ with respect to both autosomal (or X-linked) and Y-linked loci, affecting spermatozoan abnormality. The respective effects of these loci might be evaluated from the following presentation (Table 2).

It is evident that the difference between B_7 and $B_{7''}$ shows the net effect of the Y chromosome. As the percentage of abnormal spermatozoa of B_7 lies about halfway between CBA and $B_{7''}$ (or KE), the conclusion might be arrived at that the Y-linked influences are in this case of the same importance as the autosomal and/or X-linked ones.

The F_1 and B_1 generations were not taken into account in these calculations because of heterotic effects in F_1 and segregation of genes in B_1 which involve further complications.

As the B_2, B_3, B_4 and B_5 backcrosses were kept heterozygous for the albino and agouti loci it might be argued that these genes segregating in the sixth and the seventh generations have biased the results. However, no differences connected with coat colour were found to influence the character in question.

Attention was paid to the morphology of the normal spermatozoa which show differences in shape between strains (Text-fig. 2; Plate 1, figs. 1, 2). Although the exact measurements were not taken it seems that sperm head proportions in the seventh generation of backcrosses (B_7 and $B_{7''}$) are rather typical for KE strain and no differences attributable to the *Y* chromosome were noticed (Plate 1, figs. 4, 5).

Table 2. *Influence of the genetical background and of the* Y *chromosome on the percentage of abnormal spermatozoa*

Males	Source of: Autosomes and X chromosome	Source of: *Y* chromosome	Percentage of abnormal sperms
Inbred CBA	CBA	CBA	5·9
B_7 (KE × B_6)	(Approx.) KE	CBA	10·2
$B_{7''}$ (B_6 × KE)	(Approx.) KE	KE	16·1
Inbred KE	KE	KE	16·1

Text-fig. 2. Differences in shape between CBA and KE spermatozoon based on 10 spermatozoa in each strain.

(ii) *Percentage of fertilized ova*

The inbred strains used in the present experiments differ also with respect to fertilization rate. All ova were fertilized in fertile matings of the CBA males (Table 3) while previous results (Krzanowska, 1960) showed that in pure matings of KE mice about 24% of ova remain unfertilized, half of them containing spermatozoa in the perivitelline space. To test whether this inefficiency of KE sperms is also attributable to the *Y* chromosome, B_7 and $B_{7''}$ crosses were compared. Table 3 shows that in matings of $B_{7''}$ males with $B_{7''}$ or KE females the proportion of unfertilized ova (15%) is lower than in the KE strain (24%) but significantly higher than in the reciprocal B_7 (5%). Again, about half of the unfertilized ova contained one or several spermatozoa in the perivitelline space. Also about 50% of fertilized ova, at the two-blastomere stage, contained some supernumerary spermatozoa under the zona pellucida. However, no quantitative difference between the reciprocal backcrosses could be stated in this respect.

KE spermatozoa are known to be more efficient with the ova from the Outbred strain (Krzanowska, 1960, 1964) than with KE ones. Similar results were obtained

in the backcrosses. When tested with highly fertile F_1 (Outbred × KE) females (because Outbred females were not available at that time) both B_7 and $B_{7''}$ males gave a nearly normal fertilization rate (Table 3) and the difference between them was nonsignificant. However, even in this case about 50 % of unfertilized as well as fertilized ova contained supernumerary spermatozoa in the perivitelline space; in one case 20 of them were found in one two-blastomere egg.

In the CBA strain only 19 % of ova with supernumerary spermatozoa were found and never was their number higher than one per egg.

Table 3. *Fertilization rate after mating with inbred KE, CBA and the backcross males*

Males		Females		Ova in fertile copulations		
Genotype	No.	Genotype	No.	No.	% fertilized	% unfertilized containing sperms
CBA	10	CBA	14	93	100·0	—
CBA	4	KE	8	65	100·0	—
B_7 (KE × B_6)	16	KE or B_7	16	129	95·1	1·5
$B_{7''}$ (B_6 × KE)	17	KE or $B_{7''}$	17	138	85·5	7·2
KE†	10	KE	13	130	76·1	13·1
B_7 (KE × B_6)	16	F_1 (Outbred × KE)	16	136	95·0	2·9
$B_{7''}$ (B_6 × KE)	20	F_1 (Outbred × KE)	20	155	98·0	1·3
KE†	9	Outbred	9	81	92·6	3·7

† From previous investigations (Krzanowska, 1960).

(iii) *Karyotype analysis*

Chromosome preparations of KE males gave the typical picture. On well spread plates 40 chromosomes were regularly found and the *Y* chromosome could be recognized (Plate 1, fig. 6) as the shortest one. No gross abnormalities were noticed concerning its shape, although its length in relation to the first chromosome pair seemed to be somewhat shorter in KE than in CBA males.

4. DISCUSSION

There is a vast literature devoted to investigations of sperm morphology and its connections with fertility problems. Inbred strains of mice are known to differ widely in this respect (Beatty & Sharma, 1960), indicating strong genetical control. For some characters heritability estimates were made, yielding very high figures (e.g. Woolley & Beatty, 1967).

However, there are only a few reports linking sperm morphology with specified genetical loci. An outstanding example is the influence of the alleles at the T-locus on morphological abnormality and physiological inefficiency of spermatozoa (Bryson, 1944; Braden, 1960; Bennett & Dunn, 1967).

The previous analysis of the genetical background of sperm abnormality in the KE strain showed that the character is polygenically determined. The results

of the F_2 and the backcrosses between the KE and KP strains indicated that at least three genes are involved, one of them located on the Y chromosome (Krzanowska, 1966). The present data collected from the crosses between KE and CBA strains give support to the previous conclusion. The fact that in $B_{1''}$ at least one-quarter of all males (5 out of 19—Text-fig. 1) had a proportion of abnormal spermatozoa as high as in the KE strain indicates that the number of genes involved is not high, probably of the order of two (Y locus not including). Because of a great variance in the KE strain and the lack of an F_2 generation the exact analysis cannot be made. Again, the importance of the Y chromosome has been confirmed. In the backcrossing programme it became possible to separate the Y-linked factor from the other polygenes and to show that its effect remains considerable with respect not only to morphological abnormality of spermatozoa (Table 2) but also to their fertilizing capacity (Table 3).

To see whether the Y-linked factor in the KE strain is not connected with a conspicuous deficiency of that chromosome karyotype analysis was carried out. However, it failed to reveal gross abnormalities in the KE strain. The Y chromosome is rather typical and could be recognized as in other strains (Ford, 1966). Although it seemed to be somewhat shorter than in the CBA strain, this may not be very important, as according to many investigations the length of the Y chromosome is very variable. It is known to differ significantly between inbred strains of rats (Hungerford & Nowell, 1963) and between human races (Cohen, Shaw & MacCluer, 1966).

As stated earlier (Krzanowska, 1966) the linkage of the factor affecting male fertility with the Y chromosome may be important from the evolutionary point of view. Such a gene is restricted to males only, where its selective value is high and where it is exposed to strong selection. It seems reasonable to look for further Y linkages among such characters. Inbred strains are mostly suitable for these attempts because the contribution of the other genes transmitted through the female may be easily revealed even when they manifest themselves in males only.

SUMMARY

Two inbred strains of mice differing in the mean percentage of spermatozoa with abnormal heads were used: KE (16·1 %) and CBA (5·9 %). The F_1 resulting from the crosses exhibited a heterosis effect, while in the backcrosses an obvious segregation of genotypes was observed; both generations showed a reciprocal difference, depending on the source of the Y chromosome. The character of sperm head abnormality seems to be polygenically determined, one of the genes being located on chromosome Y.

Seven generations of backcrosses were performed in which the Y chromosome from CBA was introduced to the genetical background of the KE strain. In the seventh generation 10·2 % of abnormal spermatozoa were found, which is significantly lower than in the KE strain. The difference shows the net effect of the Y-linked locus. A correlated difference was found in the fertilization rate, indicating

that a factor influencing male fertility is located on chromosome *Y*. It does not seem to influence the shape of normal spermatozoan heads.

Karyotype analysis did not reveal gross abnormalities in the KE strain.

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