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The Y-chromosome-bearing sperm: a re-examination of the sex ratio in man, and new implications on the heredity of the XYY syndrome

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DEDICATION

To my parents and

my wife.

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" I remember having seen that in the sperm of man and also of a dog there were two sorts of animalacules. Seeing these, I imagined that one sort were males and the other females. "

Antoni van Leeuwenhoek
Missive 37
January 22, 1683 [1]

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Abstract:

Using the new quinacrine dihydrochloride staining technique to identify the Y chromosome, this study examined the sperm from normal (XY) men and from a known XYY man.

After demonstrating two populations of sperm (with and without an F-body) in fresh ejaculated seman samples from XY men, attempts were made to reexamine the effect of pH on the migration of these two types. No differential migration could be found at four different pH's (6.5, 7.3, 7.9, 8.4) chosen to represent the normal variation in pH during the female cycle.

Semen as well as peripheral blood were obtained from a known XYY man. A small, but significant percentage of sperm with two F-bodies were found, and these were felt to represent definite evidence of transmission of the extra Y chromosome through the meiotic division division of spermatogenesis.

Introduction:

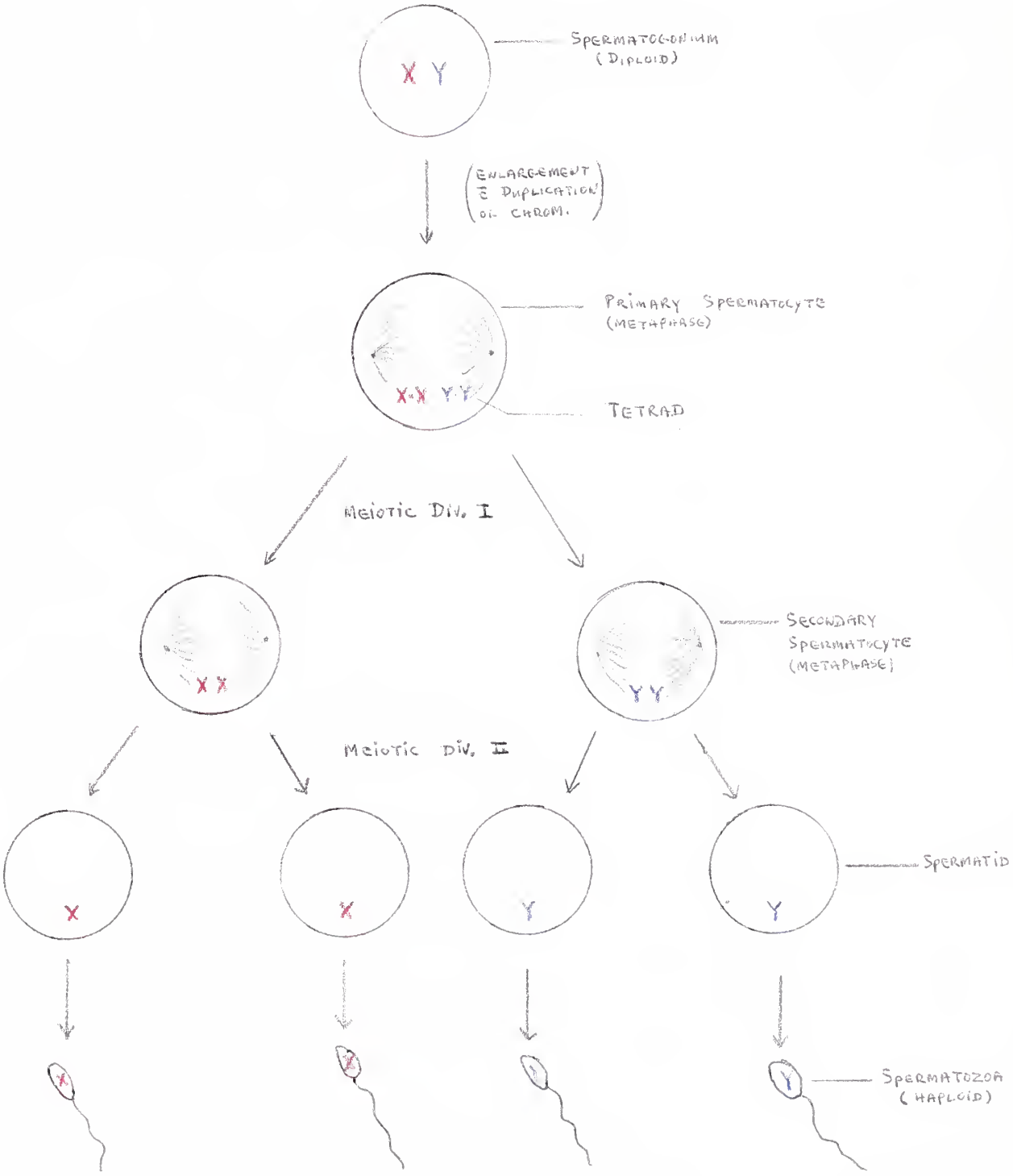
The understanding of the basic mechanism of sex determination in man grew out of knowledge acquired during the late eighteen hundreds and early nineteen hundreds on the processes of gametogenesis and chromosonal inheritance.[2] It is now generally accepted that the biologic sex of an offspring is determined at the time of fertilization, depending specifically on whether the X-bearing ovum is fertilized by an X-bearing sperm, in which case the sex will be female, or by a Y-bearing sperm, in which case the sex will be male.

Thus, it is by means of these two populations of sperm that our diploid species is provided with a mechanism for insuring the next generation of having male and female offspring. Theoretically, from what is known of the process of spermatogenesis, there should be equal numbers of X and Y sperm produced, as a result of the meiotic division. (See Fig. 1).

It has been an assumption of quantitative genetics for many years that each of these two types of sperm possess the same potential for travelling the distance to the ovum; the union of the gametes being random and independent of their specific genetic content.[3] Barring any environmental selection factor for either

of the two types of sperm, one would expect equal numbers of XX and XY zygotes resulting from an infinitely large number of fertilizations.

Fig. 1: Normal Spermatogenesis in the human male.



Sex Ratio in Normal Man:

From what has just been said, one would expect that on reviewing a large series of birth data, the sex ratio (defined here as the "secondary sex ratio" or ratio of males to females at the time of birth as differentiated from the "primary sex ratio" or the absolute ratio of male zygotes to that of female zygotes at the time of fertilization would be approximately 1:1.

As early as 1915, studies of live births, where babies were sexed macroscopically by external genitalia, demonstrated a slight preponderance of male offspring.[4] With the discovery of nuclear sexing techniques of human cells by Moore and Barr[5] in 1954, a more accurate method of sexing not only live births, but also embryos and fetuses closer to the zygote stage, became available. The secondary sex ratio is now generally accepted to show a slight preponderance of male offspring, with the usual quoted ratio as being M/F = 106/100.[6]

Utilizing the Barr Body Technique, studies have been performed on human spontaneous abortions, with the hope of obtaining a better idea of the primary sex ratio. Reports by several different authors,[7,8,9,10] have all demonstrated an even greater M/F ratio. Ratios as high

as 3.29 for collected abortive material from the total gestation period have been reported.[7] The explanation for the higher ratio for spontaneous abortion material has been that in the case of the male, the X chromosome is in the hemizygous state, and that recessive genes on the X chromosome that are incompatible with life would be expected to express themselves more often, thus fewer males being expected to live until the time of birth.[11]

As a result of these and other sex ratio studies on sexing the products of fertilization, it is clear that the primary sex ratio or the ratio of XY zygotes is greater than the 1:1 ratio that one would expect, given the conditions stated in the Introduction.

Attempts at Explanation

Over the years, many different explanations for the uneveness of the sex ratio have been offered. Related to these, has been a fervent desire of man to predetermine the sex of his own offspring.[12] The anthropologic literature abounds in descriptions of man's early methods for selecting the sex of his offspring. Most of these "theories" centered on the influence of external factors (such as the season, full moons, the influence of the tides). With the understanding of the basic anatomy and physiology of the reproductive systems, man began to search for internal factors which might

influence sex determination - e.g. sperm produced by the right testicle or ova produced by the right ovary might account for male offspring. It was not until the early nineteen hundreds with the knowledge of gametogenesis, fertilization, and the chromosomal pattern of inheritance that the foundation was laid for the modern interpretation of the sex determining mechanism.

The basic approach of twentieth century investigators of this problem has been to focus on possible inherent differences between the two populations of sperm and on factors which might predispose to one type of sperm being more successful at fertilization under certain conditions than the other type. One of the basic difficulties in these studies has been the inability of researchers to distinguish on a morphologic basis two distinct populations of sperm. It had been felt that since the Y chromosome was smaller than the X, the Y-bearing spermatozoa might therefore be expected to also be smaller. There were several reports in the early part of the century suggesting this, including Parkes[13] who claimed to find a bimodality of sperm in the man, rat and mouse. More recently Shettles[14-24] reported being able to see chromosomes wilthin the heads of mature human spermatozoa. Using a phase microscopy system, he claimed that the small-headed ones with a round centrally located mass

represented Y-bearing sperm, while the larger sperm with an elongated central mass represented X-bearing sperm. He cited evidence in a family with a pedigree of 33 males and 2 females, over the last 250 years in which, the next to the last male offspring had a preponderance of small-headed sperm.[18] Shettles has claimed that it is because the Y-bearing sperm are smaller that they reach the ovum faster, thus producing the slight excess of male offspring noted earlier.[25] Other workers have strongly criticized Shettles' work, notable among them have been Bishop,[26] Rothschild,[27] and van Duijin.[28,29] On a statistical basis alone, van Duijin has shown that no dimegaly exists among human sperm. It is important to note further that even if a dimegaly or dimorphism existed, it would not necessarily point to X-and Y- bearing sperm. There are other potential causes of duality, such as - left and right testis, live and dead spermatozoa, spermatozoa with or without cytoplasmic globule, or with and without acrosomes.[3]

Faced with this fact, that no reliable method of distinguishing the two types of sperm was available, and that no bimodality with respect to size seemed to exist, many investigators turned their attention to

searching for possible differing physical attributes of the two types. Closely related to the size differences noted above was an attempt to separate sperm on the basis of their differing specific gravities. This approach was based upon the fact that since the DNA is exceptionally heavy, X and Y - bearing sperm presumable differ in specific gravity and/or mass.[30] It has been estimated that for human spermatozoa a theoretical difference of .0001 exists for specific gravity. Experiments have been designed for utilizing animal sperm to test this possibility. Insemination with different classes separated after sedimentation showed no effect on the sex ratio.[31]

Another possible difference between X and Y sperm is with respect to their surface charge and therefore electrophoretic behavior. A change in the sex ratio might be expected in the progeny after insemination of anode - or cathode-migrating spermatozoa. The results of such experiments have been equivocal for the most part, and it is generally felt now that the sex ratio is not altered.[3]

A slightly different approach of explaining the sex ratio has been to consider the affect of the female reproductive tract on sperm as they make their

way to the ovum. Perhaps a difference in viability of the two types of sperm might exist. Furthermore, since the human female reproductive tract undergoes cyclic changes including chemical changes in the cervical mucus, consideration was given to the affect of these changes on sperm viability. Much of the stimulus for this approach came from artificial insemination studies. Kleegman,[32-34] who had an extensive experience with artificial insemination in humans, described finding a difference in the sex ratio depending on the time of insemination with respect to the time of ovulation. She has stated that if insemination or coitus occurs at the time of ovulation or a few hours before ovulation the sex of the offspring is invariably male. On the other hand, if insemination or coitus occurs two or three days prior to the estimated ovulation time or immediately postovulatory the sex of the offspring is invariably female.

Shettles, utilizing laboratory studies as well as clinical data from artificial inseminations, added further to Kleegman's findings.[12,25] He emphasized the aspects of mass and viability of the different sperm as being a possible explanation for Kleegman's findings. He hypothesized that the male-producing

spermatozoa which contains the smaller Y chromosome would have a smaller nuclear mass, and therefore, would be smaller and perhaps more motile. Thus, if insemination or coitus were to occur at exactly the time when the egg was available, the faster sperm would induce fertilization; the offspring being male. In contrast to the male-producing sperm, the female-producing spermatozoa is larger and hardier; its viability and/or its fertilizing ability may last longer. Therefore, coitus or insemination three or four days before ovulation or after ovulation would result in female offspring. This might also be influenced by changes in the cervical mucus which before and after ovulation is more hostile, making sperm migration more difficult. Under these conditions, the hardier X-bearing sperm prevail. Shettles, using his much-disputed technique for differentiating the two types of sperm, designed a set of experiments for testing the affect of acidic and basic cervical mucus on sperm migration. He found that the X sperm migrated further than the Y sperm in acidic cervical mucus. At an alkaline pH, which he claimed would be hospitable to both types of sperm, the smaller intrinsically faster Y sperm migrated further. He has further suggested that this method

may find practical application for couples wishing either male or female offspring. In a book written for the lay public, he suggests that by utilizing either acid or alkaline pre-coital douches and relating coitus to the time of ovulation, parents may influence their chances of having either boys or girls.

On the other hand, Cohen[35] using artificial insemination in relation to the time of ovulation was unable to show an alteration of the sex ratio. One investigator has reported finding an alteration of the sex ratio, but in this study a difference was found depending on whether insemination was natural or artificial. In the former females were more prevalent at ovulation time, while in the latter males occurred more frequently at that time.[36]

Thus, at the present, there appears to be no good explanation for why the sex ratio favors males. Furthermore, though there is the suggestion that X- and Y- bearing sperm have different inherent attributes and may thus be affected differently by the external environment, there are as yet no clearcut distinguishing factors known. Lastly and perhaps most important is the fact that a reliable method for identifying both types of sperm has not been available,

and thus the search for differentiating factors for these two types of sperm has become all the more difficult.

The Special Case of the XYY Syndrome:

There are several reasons for including a study of this syndrome. It is one of a few of the sex aneuploidy states in the human male that is capable of reproduction.[37] As a result of spermatogenesis, one might expect as many as four types of sperm on the basis of their sex chromosome content.[38] Perhaps a morphologic difference might exist among the classes of sperm. As has been mentioned earlier, several authors have suggested a size and mass difference in sperm of normal (XY) males. If this is true, one would expect that this would be accentuated in the case of the sperm of an XYY. Lastly, the data on offspring of XYY has led to some confusion of the heredity of this syndrome.[39]

The XYY syndrome is a rather recent addition to the cytogenetic literature, having been first noted in 1961.[40] Part of the reason for its not having been noticed earlier and perhaps for its apparent scarcity at present is probably due to the fact that it is not picked up on routine population surveys using the

Barr body test on buccal mucosa. Furthermore, there appears to be no typical phenotype as with most of the other aneuploidy states. There are no pronounced anatomical, sexual, and mental defects which facilitate diagnosis. A possible explanation for the lack of phenotypic abnormalities is that the Y chromosome is thought to have a relatively insignificant gene content. Thus an additional Y chromosome may have little effect.[41]

From the few hundred XYY males that have now been studied, there are a few features which have been described as characteristic, but by no means all-inclusive. Among these has been the finding that these individuals are often quite tall, most being over 6 feet. Growth studies have shown that as children, they usually have a growth spurt that occurs earlier than in normal (XY) males. Also, described as characteristic is the finding that XYY males often have an aggressive behavior, usually resulting in their having difficulties with the law at an early age.[42] Studies of the frequency of the XYY syndrome in prison populations as compared to the general population have demonstrated a higher incidence in the former.[42-45] Other case reports have described finding neurologic

deficits,[46-47] orthopedic abnormalities,[48] endocrinopathies,[49-50] abnormal dermatoglyphics,[51-53] hypogonadism,[54-55] as well as, an occasional association with other cytogenetic abnormalities.[56-58] It must be emphasized, however, that there are individuals afflicted with this chromosomal disorder who have none of the above abnormalities. This fact, no doubt, has affected the incidence studies of the XYY Syndrome in the general population. The occurrence of XYY is thus probably higher in the general population than the 0.02% figure cited.[39]

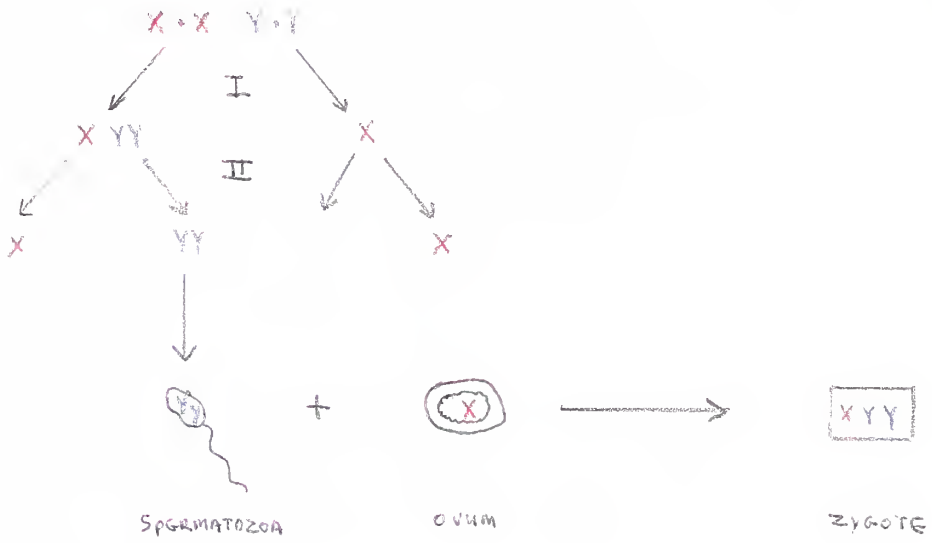
The genetics of this syndrome should be considered in several ways. First, why does it occur when the parents of such individuals are usually chromosomally normal. Second, how does the extra Y chromosomemosome behave during ehe meiotic division of spermatogenesis, when the diploid state is reduced to the haploid state. Third, if the extra Y chromosome is transmitted to sperm, how does this affect the sperm's ability to travel the distance to the egg and ultimately fertilize it. Lastly, what is the sex ratio that may be expected in the offspring of individuals with this chromosome disorder.

To explain the first point, on how such

individuals arise from chromosomally normal parents, it seems best to consider the extra Y chromosome as arising during the meiotic phase of spermatogenesis in the father, (since the extra Y could not have been contributed by the mother). Such an explanation would assume normal oogenesis in the mother, though it must be noted that a combination of meiotic defects during gametogenesis in both parents could also account for XYY offspring; this mechanism however would seem less likely from a statistical standpoint. In any case, it is the non-disjunctive event occurring in the father that is the essential fact. This could theoretically occur at three different levels (See Fig. 2,3, and 4).

Fig. 2: Hypothetical mechanisms for the origin of XYY sons from chromosomally normal parents - Non-disjunction at meiotic division I of spermatogenesis in father.

1.)



2.)

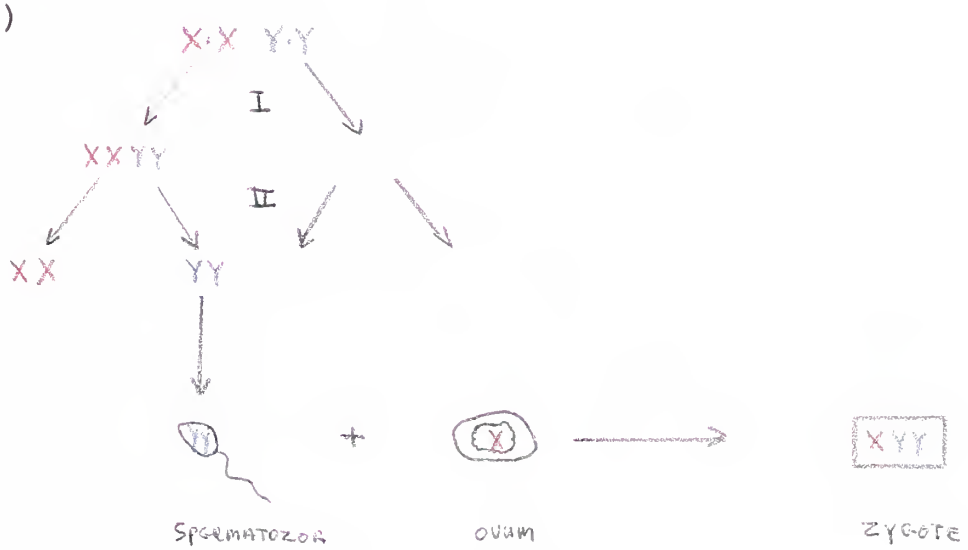


Fig. 3: Hypothetical mechanisms for the origin of XYY sons from chromosomally normal parents - Non-disjunction at meiotic division II of spermatogenesis in father.

3.)

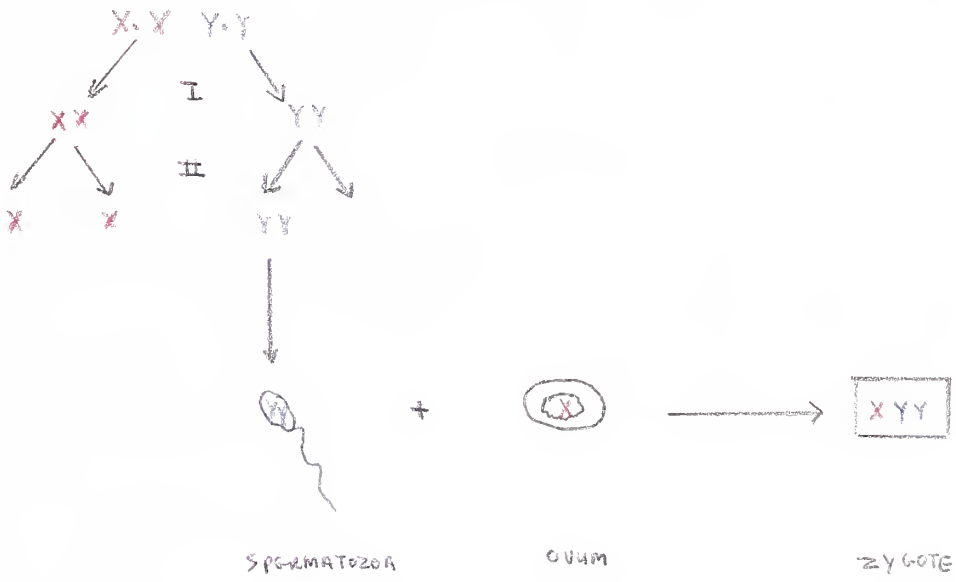
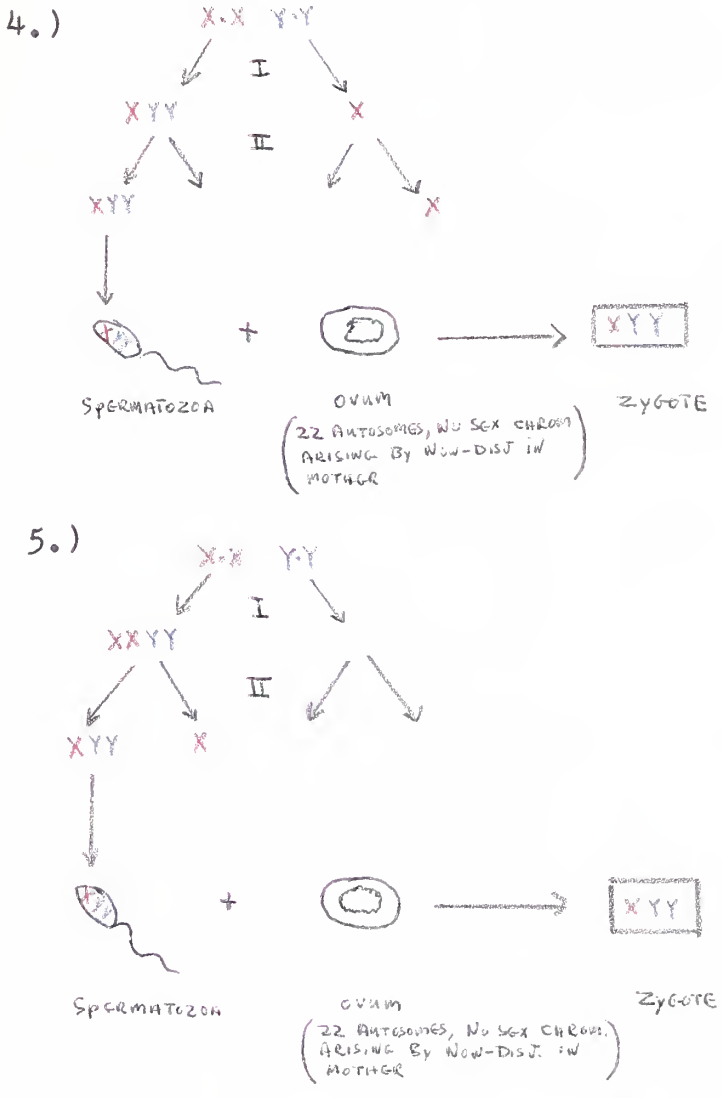


Fig. 4: Hypothetical mechanisms for the origin of XYY sons from chromosomally normal parents - Non-disjunction in both mother and father



Note: There is one other mechanism that may be proposed as causing XYY individuals. This would involve non-disjunction during the mitotic division of the spermatogonia. Proposed by Townes to account for the one known case of XYYY.[81]

The most probable mechanism would most likely be non-disjunction at either meiosis I or II resulting in the formation of YY sperm. These on fertilizing X-bearing ova would result in XYY offspring (See mechanisms 1, 2, and 3, Fig. 2 and 3). It is interesting at this point to consider the odds of a YY sperm produced by an isolated accident of meiotic mechanics, as eventually succeeding in fertilizing the egg. This is especially true when one considers that: 1. Such a sperm or sperms would be only a small proportion of the millions of normal sperm; 2. Such a sperm should be larger and have greater mass than the normal sperm carrying only one sex chromosome. Furthermore, if one considers that the incidence is probably around 0.02% or greater, one must theorize that such a non-disjunctional event must be occurring quite frequently during the normal process of spermatogenesis in all individuals.

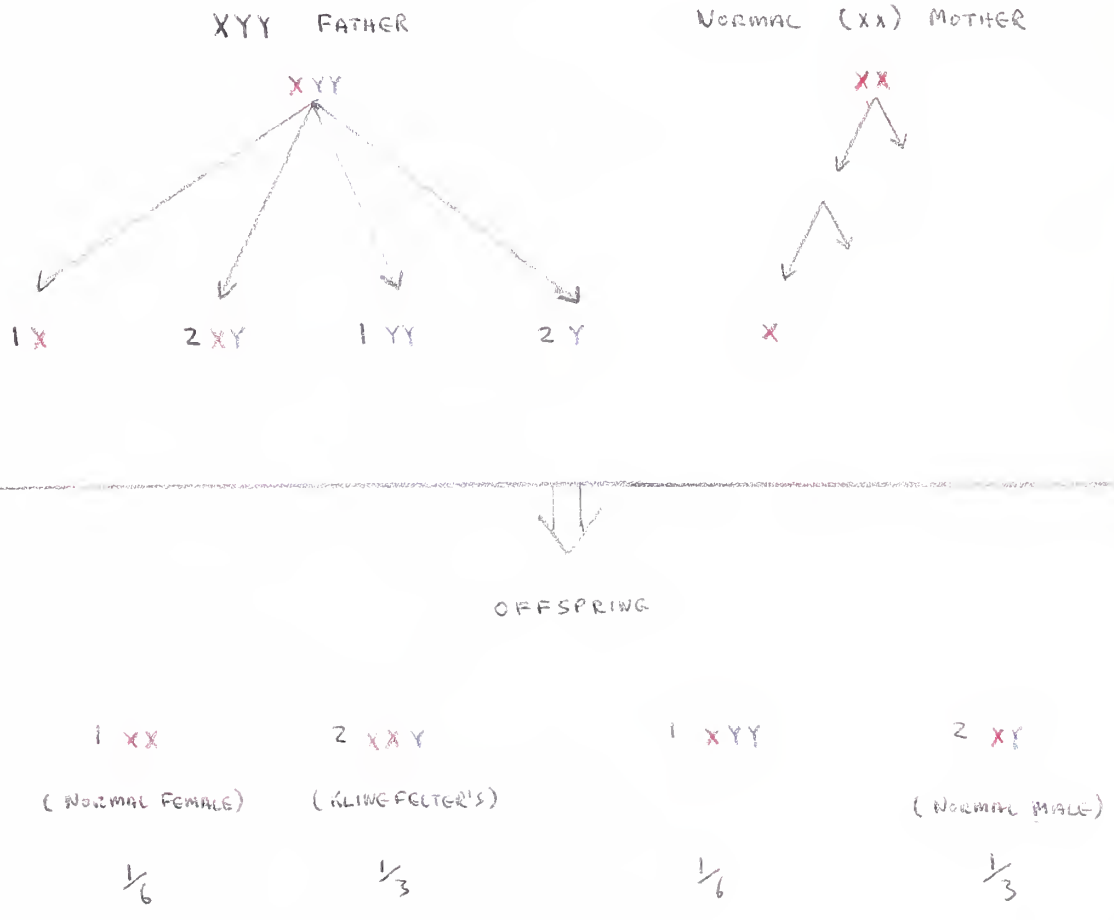
The next question concerning the genetics of the XYY Syndrome involves the affected individual himself. Since an XYY is most often capable of producing offspring, one must ask, what happens to the extra Y chromosome during the meiotic divisions of spermatogenesis? Can the extra Y chromosome be transmitted

to yet another generation? The early approach to these questions was to look at the karyotypes of offspring of these individuals. This was done by Sandberg in the first reported case of an XYY individual.[40,41] He reported two children with demonstrable chromosomal abnormalities; one daughter with an XX/XO mosaic karyotype who had typical Turner's features, and a second daughter with a G trisomy who had typical features of mongolism. He suggested that perhaps this family had a tendency toward non-disjunction. There were however no sex chromosome aneuploidy states in the children of this individual. Theoretically, one would expect that if the extra Y chromosome segregates randomly, there would be at least four types of sex chromosome combinations produced in the sperm of such an individual.[39] Thus, if these sperm are capable of fertilizing an ovum from a normal oogenesis in a normal (XX) mother there should be four types of individuals produced, including two types of aneuploid states - XXY and XYY. (See Fig. 5). Other investigators studying karyotypes of offspring of XYY men also have been unable to find XXY and XYY children. To date there has been only one report of an XYY father having an XYY son. (Paternity was demonstrated by red blood cell

antigen comparison in this case.)[59] As far as I know there have been no reports of XXY offspring arising from XYY fathers. Of course, part of the explanation for the absence of these two types may be because too few XYY individuals have been identified, and still fewer of the known XYY men have children who have been karyotyped.

The problem posed by the above findings has prompted several investigators[38,60-64] to study meiosis in testicular biopsies.

Fig. 5: XXY offspring. The four possible zygotes resulting from a union of an XXY male and a normal XX female, if segregation of the X and two presumed Y chromosomes is random. (Adapted from Parker, C.E., et al. Amer. J. of Med. 47, 801 (1969)



of known XYY men, with the hope of being able to follow the sex chromosomes through the various stages of spermatogenesis. Thompson et al.[38] were the first to report on such studies in an XYY man. They found that on karyotyping thirty-nine of the secondary spermatocytes cultivated from testicular biopsy material, thirty-nine of the secondary spermatocytes cultivated from testicular biopsy material, thirty-four of the cells contained 23 chromosomes, while the other five contained 24. Of the spermatogonial metaphases examined, none were found to have two Y chromosomes. Similarly, of the one hundred and fifty-five first-meiotic metaphase figures examined, they found none containing two Y chromosomes.

They interpreted these findings as suggesting selection toward chromosomally normal spermatocytes before the meiotic division. Furthermore, they proposed that a mechanism may exist whereby the extra Y chromosome is eliminated prior to spermatocyte formation.[38,60] It is known that such a mechanism of sex chromosome elimination occurs in at least one species in the animal kingdom. In Microtus oregom, the creeping vole, selective elimination of a sex chromosome during meiosis occurs as a natural

phenomenon. In this species the male, which has an 18,XY chromosome complement, selectively eliminates the X prior to spermatocyte formation, resulting in some sperm with a single Y and some without a sex chromosome.[65]

Other investigators have shown that a YY bivalent can be found in meiotic studies of XYY men, though as in Thompson"s work these seem to be infrequent.[61-64]

Another possible explanation for the apparent infrequent transmission of the extra Y comes from work done on DNA-replication studies. It is known that the DNA replication pattern of the two Y chromosomes differs slightly.[66-67] Tettenborn, et al.[64] have suggested that under such circumstances a preferential anaphase lag of one of the Y chromosomes during spermatogonial divisions might lead to elimination of an extra Y chromosome.

As far as I know, there have been no comprehensive studies done on sperm morphology in this syndrome. About the only fact noted in previous investigations of XYY men, is that these individuals tend to have oligospermia and often have a preponderance of abnormal forms.[68] There have been no reports to my

knowledge of possible segregation of sperm into different sub-populations on the basis of size and shape. As in the sperm of normal (XY) men, investigations have been hampered by the lack of a method for distinguishing the types of sperm on the basis of their sex chromosome content.

Human Spermatozoan Populations - Problem of Morphology:

Since the time of Leeuwenhoek, biologists have sought a means of differentiating X- from Y- bearing sperm. With each succeeding technologic development in microscopy, there was new hope that a method would be found. Many fundamental questions in biology have been unanswerable because no such method was available. The question of the sex ratio in normal (XY) man and the factors which influence it, as well as the question of what happens to the extra Y chromosome during XYY spermatogenesis with its implication on the heredity of this syndrome could be better approached if X- and Y- bearing sperm could be identified.

It is now apparent that light microscopy does not offer an answer.[29] Experimentation with various staining techniques has not been helpful. Unfortunately, nuclear stains like the Barr Body technique, which is so helpful is diploid somatic cells, is of no use in the haploid sperm.

Shettles, using a phase microscopy system, claimed the ability to distinguish X-bearing from Y-bearing sperm on the basis of what he called the size and shape of the centrally located chromosomes.[14-24] His method has been strongly criticized.[26-29] It is generally agreed now that the "centrally located chromosomes" that Shettles claimed to see were most likely phase contrast optical aberration.

Despite the development of electron microscopy, the two types of sperm could not be distinguished.[69-70] E.M. studies on human sperm have shown that distinct chromosomes are not visible, and that the nuclear material is tightly packed into a dense chromatin mass.[69]

In 1969, Zech[71] reported that, when the DNA-binding fluorochrome, quinacrine mustard, was used to stain metaphase plates from human blood cultures, the Y chromosome could be easily recognized by an "especially bright fluorescence of the posterior part of the long arm." This finding has since been confirmed by other investigators.[72,73] It is now generally agreed that there is a preferential uptake of the DNA-binding fluorochrome by different chromosome regions in a constant and reproducible pattern.

The biochemical explanation for this binding was first attributed to quinacrine mustard's alkylating properties. It is known that quinacrine mustand binds to the N-7 atom of quanine. Therefore, it was thought that the stain was picking out segments of DNA rich in quanine. However, this theory has not held up to testing. It is now felt that the areas of chromosomes stained by quinacrine mustard consist of heterochromatin.[83]

Of the 46 chromosomes in man, it has been found that chromosomes 3, D, and &, take up the fluorochrome most selectively. The uptake is especially conspicuous in the human Y chromosome.[74,75]

This finding has prompted other investigators to look for "fluorescent bodies" within interphase cells. Pearson et al.[72] have reported finding a single fluorescent body within male buccal smears. They tested the validity of their technique by presenting coded buccal smears independently to two observers. A total of twenty-four smears were used, fourteen from normal males, and ten from normal females. Each misclassified one slide, which was noted later to be of poor technical quality. Pearson's group also reported finding a single fluorescent body within cultured lymphocytes and fibroblasts from

normal (XY) males. Thus, it would seem that this method might provide a means for determing the genetic sex in interphase nuclei.

The quinacrine fluorochromes have since been used to stain human spermatozoa. Barlow and Vosa[76] in the summer of 1970, reported finding a single fluorescent body in approximately 42% of one thousand sperm examined from two normal (XY) males. They proposed that those sperm containing a fluorescent body (F-body) were X-bearing sperm. Pearson and Bobrow[77] used this staining technique on testicular biopsy material from 3 normal adult males. They found that on counting 1420 spermatids - 733 (51.6%) contained no F-body, 666 (46.9%) contained one F-body and 21 (14%) contained 2 F-bodies.

It is with this staining technique that I hope to examine some of the questions posed earlier.

Purpose of this Study:

The purpose of this study was twofold. The first phase of this research attempted to reexamine some of the factors which might influence the sex ratio in normal (XY) men. I have chosen to study the affect of pH on the migration of X-bearing and Y-bearing sperm through a pre-determined distance within a selected period of time. Of the several possible factors which might influence the sex ratio, the affect of pH seemed to be worthy of reconsideration. (Since the pH of cervical mucus is known to vary 2 - 2.5 units during the human female cycle,[78] and in view of Kleegman's clinical findings (artificial insemination) and Shettles' experimental observations. By utilizing the quinacrine staining technique, it should be possible to distinguish between X and Y sperm. Thus, if pH could be found to cause a differential migration of the two types at different pH's, one might be able to better understand the affect of pH on the sex ratio.

The second phase of this research has been to examine spermatozoa from a known XYY man, with the hope of obtaining a better idea of the frequency of transmission of the extra Y chromosome. Such a

method should allow one to acquire a more precise picture of "the products of meiosis" in this syndrome, especially in light of the fact that family studies are limited in number, and testicular biopsies can only give a partial picture.

Materials and Methods:

Basic to both phases of this research was developing a working technique for staining sperm, as well as a fluorescent microscopy system for viewing samples.

Because of the rather limited supply of quinacrine mustard available, quinacrine dihydrochloride (Atabrine, Winthrop) was used for staining. The successful use of this compound for staining for the Y chromosome had been described earlier.[72,79] Slides were prepared as follows. Sample to be analyzed was placed on slide and allowed to air dry for 15 min. The slide was then treated with absolute alcohol for 1 min., 0.5% aqueous solution of quinacrine dihydrochloide for 3 min., rinsed briefly in tap water, rinsed a second time in deionized distilled water, and finally mounted with tris-malleate buffer, pH 5.6. This method is essentially like Pearson's[77] except for the last step.

All slides were examined with a Leitz Ortholux microscope utilizing an HBO 200 light source, BG 12 excitor filter, a 530 nm. barrier filter, as well as an oil fluorescence condenser, and oil objective (Leitz 100). All slides were read within six hours

of staining to avoid fading of fluorescence. Photomicrographs were taken with a Nikon camera, using high speed Ektachrome film.

A.) Sex Ratio Study:

Freshly ejaculated semen samples were obtained from human donors. Each sample was examined for sperm count, motility, and morphology. Those sperm which did not meet the criteria for normal males in all three categories were excluded.

Studies were done at first on smears prepared directly from semen samples and treated as above. Slides were then scanned for 100 successive sperm, noting the total number containing F-bodies.

In order to test the effect of pH on the migration of X and Y sperm, a technique was devised whereby capillary tubes filled with Tyrodes solution adjusted to different pH's were exposed for a period of time to a semen pool. (See diagram below).

Stock solutions of Tyrodes solution adjusted to pH 7.3, 7.9, and 8.4 were prepared and checked periodically. These values were chosen to represent the range normally found in human cervical mucus during a typical cycle. Alkaline-free non-corrosive 1.1. mm. I.D. capillary tubes, capped at one end, were filled 1/5 (20 mm.) of their length with the three different solutions. The tubes were then placed vertically into each semen sample. After 1-½ hours the tubes were removed and the distal 5 mm of each was broken off and discarded. The remaining contents of the capillary tube were blown onto a slide and then treated as described above. Each slide was then scanned for 100 successive sperm and the total number containing F-bodies noted.

B.) XYY Study:

H.J.D. is a 38 year old, white, male who is presently a patient in a state mental hospital in Connecticut. During the summer of 1968 he had chromosome studies done at the request of his physician and was noted to have an Xyy karyotype.

Review of the patien's history reveals several features suggestive of this syndrome. At the age of fourteen he was sent to reform school after several

minor confrontations with the law. This was followed by several prison sentences for robberies, including one attempted rape. Since 1966 he has been hospitalized in the maximum security section of a state mental hospital. Review of his family history, reveals phenotypically normal parents, as well as phenotypically normal sibs (2 brothers and 1 sister). He has never had children. Chromosomal studies have not been done on parents or sibs.

Physical examination is for the most part normal except for his height (6'3") which would be consistent with XYY Syndrome.

This patient is one of five known XYY individuals in Connecticut who have been karyotyped by the Yale-New Haven Cytogentics Lab. The other four were not available for study; three of the five being children, while the other one was serving a prison sentence in Massachusetts.

H.J.D. agreed to be a subject in this research. A blood sample for fluorescence karyotyping as well as five semen samples were obtained from him.

Results:

A.) Normal (XY) men:

With the quinacrine dihydrochloride stain, the sperm head was seen as consisting of an anterior region which stained poorly and a posterior region which stained with a yellowish-green fluorescence. The F-body, when present, appeared as a much brighter, yellowish-white, fluorescent spot usually located along the boundary between these two regions (See photo 1 and 2).

In accord with the findings of Barlow and Vosa,[76] the percentage of sperm possessing an F-body ranged from 36% - 49% in smears prepared directly from fresh ejaculates. In no case did a sperm have more than one F-body (See Table 1).

Fifteen samples were analyzed for possible differential migration of sperm containing an F-body, into Tyrode's solution at the three pH's mentioned earlier. Small changes in pH did not influence the ratio of sperm with F-bodies migrating into the tubes. In order to evaluate possible fluctuations in sperm ratio at a more acid pH, six additional samples were analyzed in Tyrode's solution adjusted to pH 6.5. There was no significant change in the ratio of sperm

with F-bodies. Again, no sperm were found with more than one F-body. (See Table 1).

B.) XYY man:

Chromosome preparations from a peripheral blood sample of the subject, H.J.D., were made and treated with the quinacrine dihydrochloride stain. Photo 3 shows chromosomes have dull fluorescence, but with especially bright, white fluorescence on the distal portion of the long arm of the Y chromosomes.

Examination of the semen sample under light microscopy revealed a sperm count of 15 million per ml., motility of 50%, and less than 25% abnormal forms. These criteria together with an average sample volume of 1.5 ml. would suggest subfertility in this patient.

Smears were made from semen samples and stained with quinacrine dihydrochloride. One thousand spermatozoa from the XYY subject were counted. Of these, approximately 70% contained one F-body, approximately 5% contained two F-bodies, while the remaining sperm contained no obvious F-bodies. In the XY data above, it was noted that 36%-49% of the sperm examined had one F-body and no sperm possessed more than one F-body (5700 sperm were examined). When two F-bodies were present they appeared as distinct fluorescent

spots which tended to line up in the longitudinal axis of the sperm head. Usually, one of the two was brighter; this perhaps was due to their relative depth within the sperm head. The size of the F-bodies within a sperm head were approximately equal. They were located relatively close together, but they did not appear contiguous. A typical sperm with two F-bodies is shown in Photo 4. It should be noted that sperm with two F-bodies did not appear grossly abnormal in any way. These sperm did not appear larger than sperm possessing one F-body or sperm with no apparent F-bodies. Thus there appeared to be no distinguishing characteristics of the sperm on the basis of their chromosomal makeup. Furthermore, since no technique is at present available for selectively staining the X chromosome in sperm, one cannot be certain of the percentage carrying the XY chromosomal complement. These are probably represented in the group containing one F-body.

TABLE I. Percentage of Sperm from Normal (XY) Men Containing a Single F-body

	Fresh Ejaculate	pH 6.5	pH 7.3	pH 7.9	pH 8.4
Mean	42.6	43.0	41.9	42.9	41.7
1 S.D.	± 4.2	± 6.6	± 2.9	± 6.2	± 3.6
Number of Samples*	6	6	15	15	15

* 100 Sperm Counted in Each Sample.

Note: In none of the 5700 sperm examined were more than 1 F-body found.

Discussion:

Before discussing the results obtained from these two studies, it is worhtwhile to reconsider the basic assumption that has been made, namely that the F-body represents the cell possessing the Y chromosome. The basis for the assumption rests on studies of meiotic and mitotic metaphases in which the distal end of the long arm of the Y chromosome was shown to fluoresce more brightly than any other chromosome.[71-74] The fact that in interphase cells (buccal mucosa and cultured fibroblasts) from normal XY males only one F-body was found, while from normal females no F-body was found, strongly suggests the reliability of this method in sexing cells.[72]

(As a personal observation, I might add that my own experience in examing various interphase cells - amniotic fluid cells, buccal mucosal cells, cultured fibroblasts, and lymphocytes - from both XX and XY individuals has not yielded clearcut results. While sex determination could often be made on these cells, at times interpretation was difficult, because more than one fluorescent point was found. The determination of the"true F-body" often could not be made in these cells. Whether these represent chromosome

3 or D, or artifact, is not clear.)

In contrast to the interphase cells, I found the staining reaction with quinacrine dihydrochloride to be quite characteristic in sperm. When an F-body was seen in sperm, it appeared as a distinct fluorescent spot with a bright, yellowish-white fluorescence setting it apart from the yellowish-green fluorescence of the sperm head. The F-body could be discerned whether it appeared in the relatively non-fluorescent, anterior region of the sperm head, or against the background of yellowish-green fluorescence in the posterior region of the sperm head. At no times did problems arise as with the interphase cells. Of the 5700 sperm examined from XY men, none were found to contain more than one F-body. The only sperm found to contain two F-bodies were found in the study of XYY sperm. In this case the two F-bodies appeared to have the same dimensions and staining reaction (one F-body usually appeared brighter, however; this probably being due to the relative depth within the three dimensional sperm). It is upon these observations that the interpretation of the results is based.

A.) Normal (XY) men:

The results found on studying semen smears agree with those reported by Barlow and Vosa.[76] They suggested that the reason for finding fewer than 50% of the sperm with a single F-body was because of the difficulty of resolving the F-body when it lies within the dense chromatin at the base of the head. In their study which was a report of quinacrine staining of spermatozoa from two normal (XY) men, they noted that the ratio of sperm possessing an F-body was higher when quinacrine mustard was used than when quinacrine dihydrochloride was used (45.1% of 567 sperm with the former, 40.2% of 513 sperm with the latter.) If one wishes to accept the theory that equal numbers of the X and Y gametes are produced, these results would support this theory.

The results of the study of migration of sperm through acidic and basic Tyrode's solution suggest that there is no differential migration of X-bearing and Y-bearing sperm with respect to pH. This contradicts Shettles[25] experimental results in which he claimed the X-bearing sperm migrated further in an acidic media, and also leads one to doubt the sex selective value of the acidic and basic pre-coital

douches, that he has advocated. The recent findings of Cohen[35] with artificial insemination studies in which he showed no change in the sex of the offspring with respect to ovulation time casts doubt on Kleegman's findings mentioned earlier.[32-34] Thus, on both clinical and experimental grounds it appears unlikely that X and Y sperm can be differentiated on the basis of migration through fluids of varying pH's.

B. XYY man:

The finding of sperm with two F-bodies in an XYY man, strongly suggests that Xyy men are capable of transmitting the extra Y chromosome to their offspring. Family studies have not been helpful in this regard with only one recorded case of an XYY father and an XYY son.[59] The testicular biopsy studies[38,60-64] have been somewhat limited, since the technique is rather difficult, the sample size of cells examined has been rather small, and the later stages of spermatogenesis have not been adequately followed.

The finding of a significantly larger percentage of sperm with a single F-body as compared to the XY data, seems best explained by the fact that many of these sperm may also contain an X. As noted in Fig. 5, the XY gamete should be produced during spermato-

genesis in an XYY man. Since no technique is presently available for selectively staining the X chromosome in sperm, the existance of an XY sperm cannot be proved. As mentioned earlier, there is at present no family data to support the existence of an XY sperm (XXY children from XYY fathers).

From these studies, the suggestion by Thompson et al.[38] that there is a mechanism to eliminate the extra Y chrom in XYY meiosis seems doubltful. Similarly, Tettenborn's[64] theory (preferential anaphase lag of one of the Y chromosomes during spermatogonial divisions), though not contradicted by these results, would seem at least less than a complete elimination of the extra Y. Rather than considering the meiotic stage as providing an elimination mechanism, perhaps the selectivity lies in the sperm's journey to the egg, and in the sperm's fitness" to get there first.[81]

(Personal observation. Sperm from an XYY man are now being tested with the capillary tube method mentioned earlier. Sperm are allowed to migrate into Tyrode's solution, pH 7.9. At the end of an hour and a half, capillary tube removed from semen pool and broken into an upper and lower segment of equal length. The contents

of each being emptied onto separate slides and then examined with quinacrine staining technique. Preliminary results have failed to show sperm with two F-bodies in either segment, in contrast to the 5% figure found in smears. Not enough data has been gathered at this time to make any statistical significant conclusions. Furthermore, since the XY-bearing sperm cannot be differentiated from the Y-bearing sperm (both probably being contained in the group of sperm with a single F-body), it would be difficult to draw any conclusions about the migrating ability of sperm possessing an extra sex chromosome.).

Conclusions:

1.) This is the largest study reported to date on sperm from normal (XY) men stained with the quinacrine technique. A total of 5700 sperm were examined. Of these approximately 42% possessed a single F-body, which is thought to represent Y-bearing sperm. The remaining sperm contained no obvious F-body. At no time were sperm found to contain two or more F-bodies. The figure of 42% is less than the 50% figure one may have expected, but because of the probable limitations of the technique, there is the implication that an equal number of X-bearing and Y-bearing sperm are probably produced during normal (XY) spermatogenesis.

2.) Small changes in pH did not influence the ratio of sperm with F-bodies migrating into the capillary tubes. This implies that X and Y bearing sperm have equal ability to migrate through solutions of different pH, and therefore contradicts Shettles' findings.[26] This casts serious doubt on the potential sex determining role of cervical mucus of different pH found in the normal female cycle, and also raises the question of the efficacy of pre-coital douches of different pH in altering the sex ratio.

3.) The finding of a small but significant number of sperm possessing two F-bodies in semen samples from an XYY man gives definite evidence of the transmission of the extra Y chromosome through the meiotic division of spermatogenesis, and at the very least shows the potential of these individuals to in turn produce XYY sons. It seems doubtful that any special mechanism exists in XYY men for disposing of the extra Y chromosome prior or during meiosis.

4.) There is the suggestion that a selection against the XY-bearing and YY-bearing sperm produced by these individuals may exist not at meiosis; but in the sperm's journey to the egg, and in the sperm's "fitness" to get there first.

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APPENDIX

(Photomicographs)

Photo. 1: Sperm fron a normal (XY) man stained with quinacrine dihydrochloride not showing an F-body. Note posterior staining is prominent, anterior region is poor.

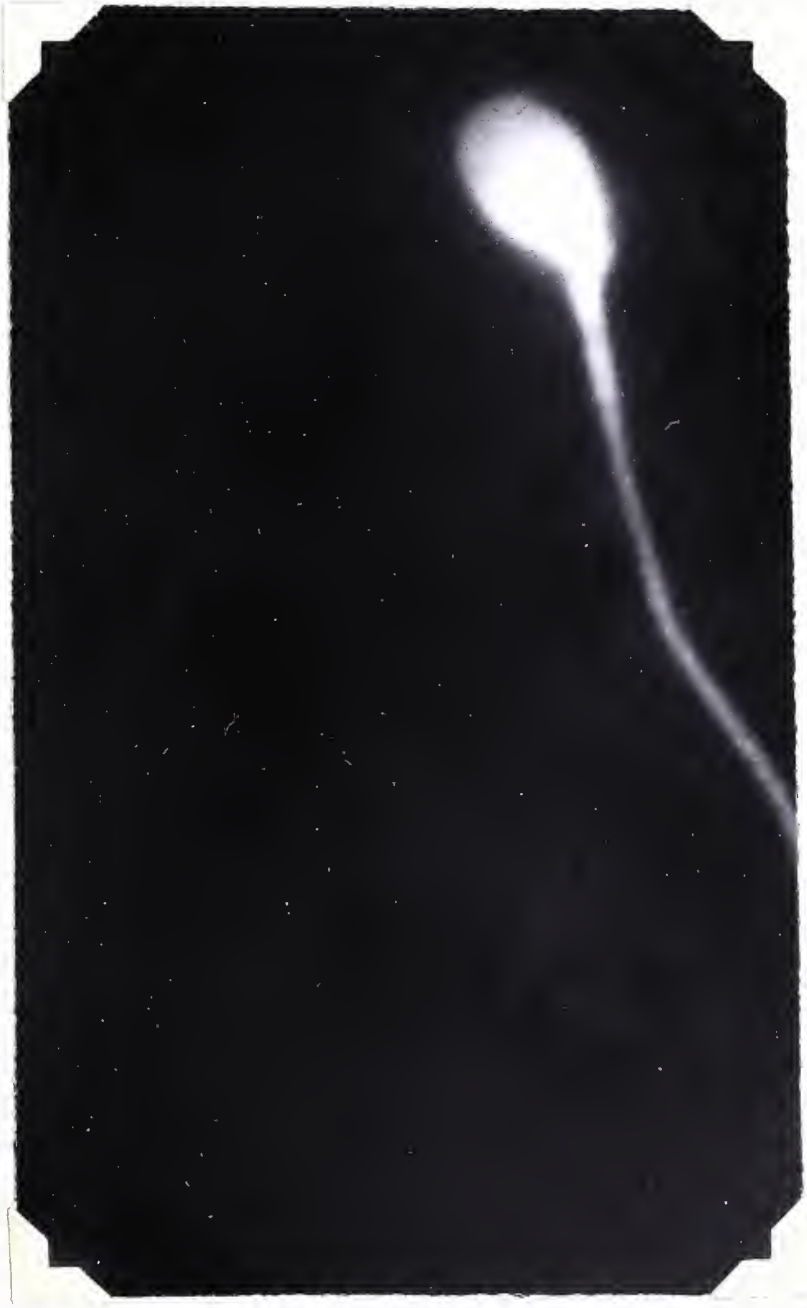


Photo. 3: Chromosomes prepared from peripheral blood sample of XYY man stained with quinacrine dihydrochloride. Chromosomes can be seen to take up stain, some more than others. The two Y chromosomes are shown.

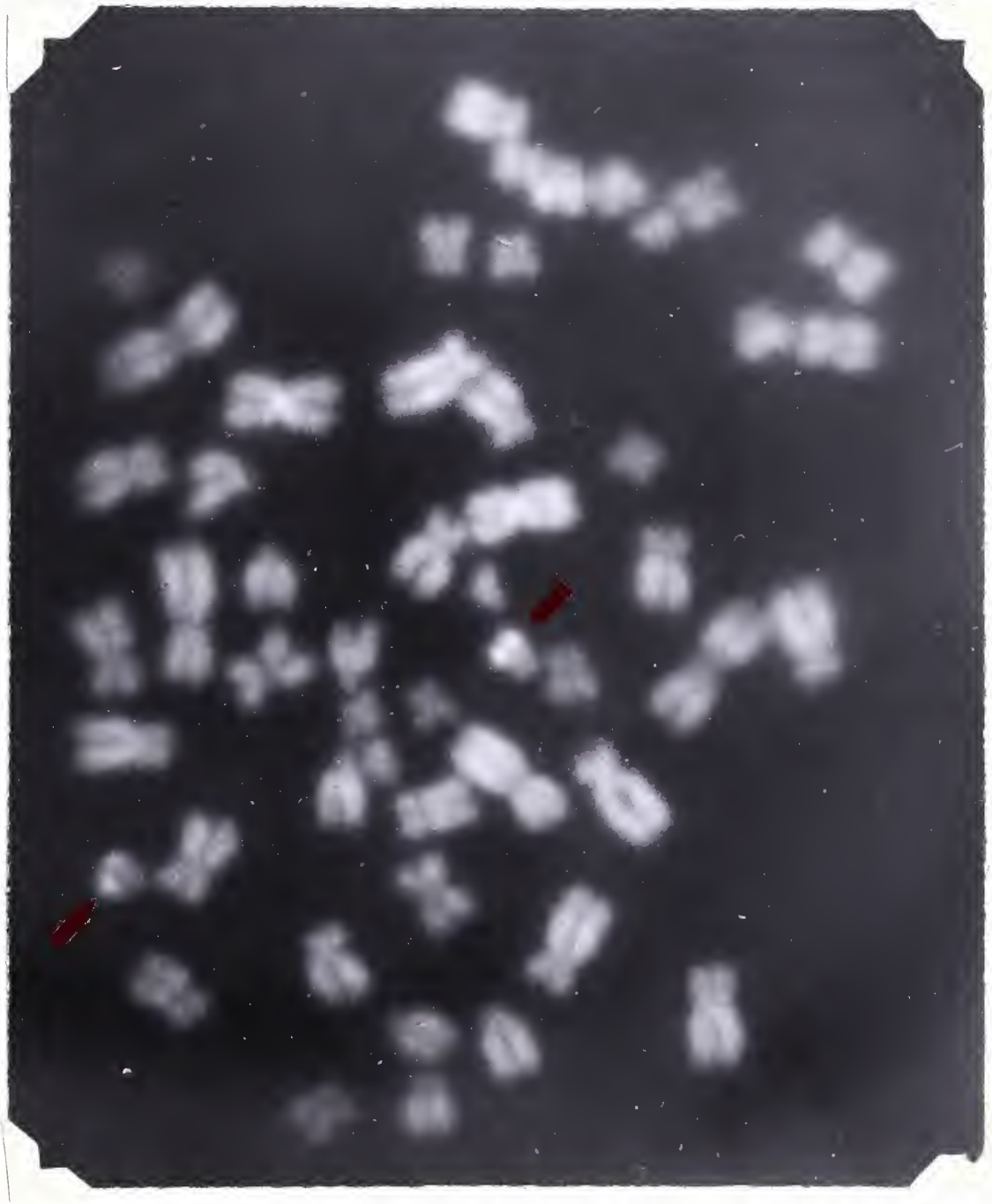
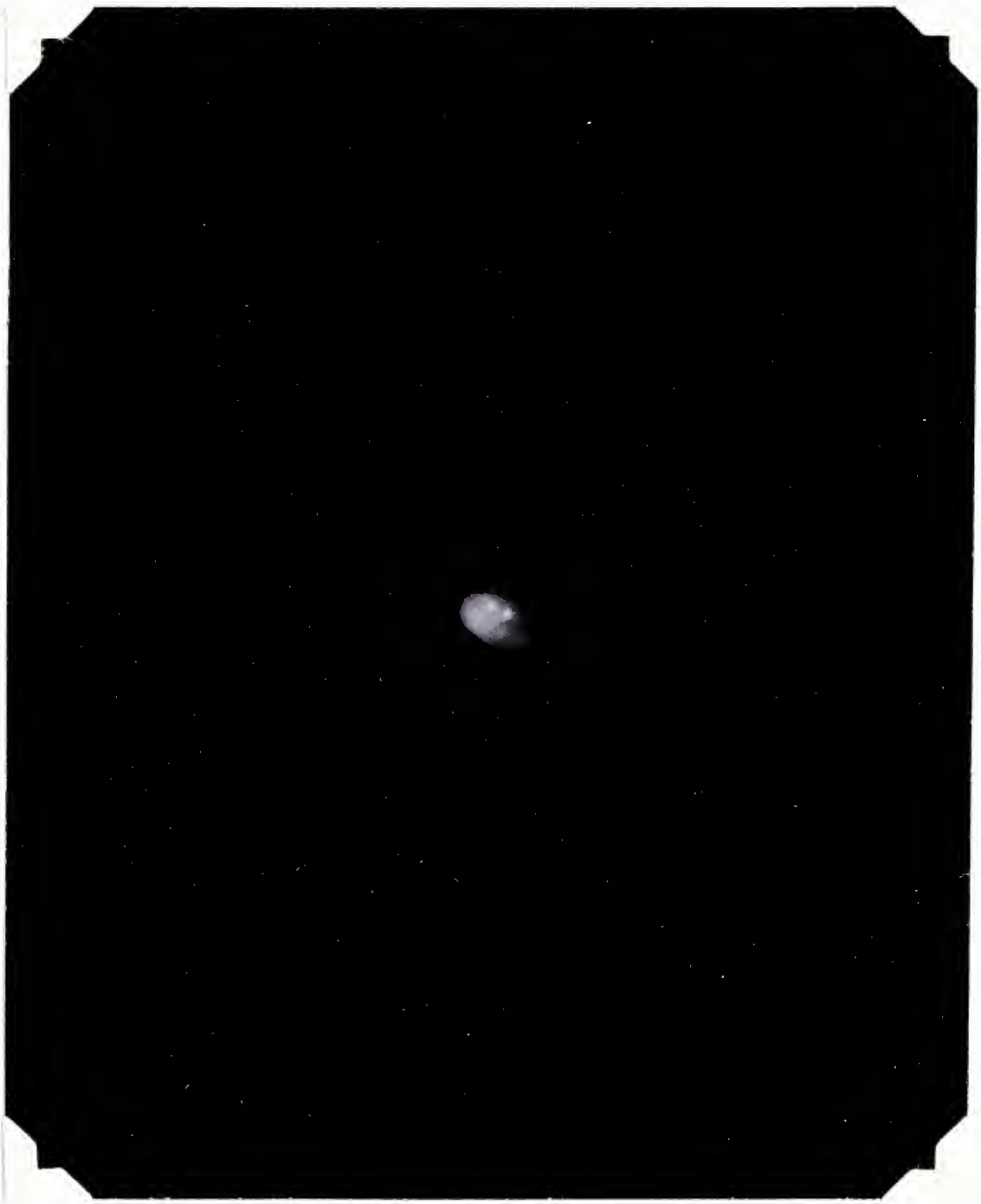


Photo. 4: Sperm from XYY male stained with quinacrine dihydrochloride. Two F-bodies can be seen in the longitudinal axis of the sperm head.



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