Sporadic Cases of Hemophilia and the Question of a Possible Sex Difference in Mutation Rates

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RARE SEX-LINKED GENES in man provide unique information on the sex ratio of mutation rates. Mutants on the X chromosome are transmitted from sperm to carrier daughters and from eggs to carrier daughters and affected sons, which appear as sporadic cases. Therefore, the proportion of cases that are sporadic depends on the relative mutation rates in egg and sperm. A completely penetrant sex-linked dominant gene would be ideal for these studies, but unfortunately none has been discovered in man. Two recessives, hemophilia and Duchenne muscular dystrophy, are sufficiently deleterious for an appreciable fraction of cases to be mutants, but common enough so that the proportion of sporadic cases can be estimated with some precision.

Contrary to an earlier suggestion by Haldane (1956), mutations to sex-linked Duchenne muscular dystrophy arise with approximately equal frequency in the two sexes (Cheeseman *et al.*, 1958; Smith and Kilpatrick, 1958; Morton and Chung, 1959). Haldane (1947) also proposed that the hemophilia locus mutates more frequently in sperm than in eggs, perhaps ten times as frequently, and this conclusion has been widely quoted (e. g., Stern, 1960, p. 461; Roberts, 1959, p. 97). He drew his evidence from an attempt to discriminate heterozygous carriers by Burker's coagulation-time method and from a segregation analysis of isolated and familial cases. We shall now show that this conclusion is unwarranted, and that the evidence is entirely consistent with the hypothesis of equal mutation rates in eggs and sperm.

CLOTTING TIME OF MOTHERS OF ISOLATED CASES

Haldane (1935) demonstrated that the proportion of affected males that are sporadic mutants if there is an equilibrium between selection and mutation is

$$x = \frac{m}{2 + v/u}$$

where m is the selection coefficient against affected males, v is the mutation rate in sperm, and u is the mutation rate in eggs. For hemophilia the selection coefficient m has been estimated to be as high as .75 (Haldane, 1935) and as low as .43 (Andreassen, 1943). Haldane (1947) pointed out that the lower value is biased by selection of fertile affected ancestors and by the use of immature brothers as a control. If mutation rates are the same in egg and sperm ($u = v$), then the proportion of sporadic cases should be $x = m/3$, or be-

Received Aug. 17, 1961.

tween .25 and .15. If the proportion of sporadic cases in the population is less than this, the mutation rate may be greater in sperm than in eggs, the sex ratio of the mutation rates being

$$v/u = m/x - 2.$$

Haldane's first argument for a deficiency of sporadic cases is based on five isolated probands studied by Andreassen (1943). All of the mothers of these cases were thought to be carriers from their coagulation time. If in fact they were carriers, this would indicate a low frequency of sporadic cases. For the probability that an isolated case with normal brothers have a carrier mother is

$$\frac{(1 - x)\ (1/2)^n}{(1 - x)\ (1/2)^n + x} = \frac{1 - x}{1 - x + 2^n x.}$$

Andreassen's five cases had 0, 0, 1, 2, 3, normal brothers, so the probability that all had carrier mothers is

$$\frac{(1 - x)^5}{(1 + x)(1 + 3x)(1 + 7x)}$$

which is .04 for x=.25 and .13 for x=.15. Because of uncertainty about the true value of x, this provides no real evidence for a higher mutation rate in sperm than in eggs, even if the mothers were in fact carriers. Moreover, Merskey and Macfarlane (1951) were unable to confirm Andreassen's claim that carriers usually show a prolonged coagulation-time by the Burker method. None of his five possible carriers had a beginning or end point outside the range of their eighteen control females. They note that the Burker method is "crude and the end-points are not very definite." Andreassen neglected to verify his claim to distinguish carriers by a blind test with normal controls. Using more sensitive tests of prothrombin consumption time and titration of antihemophiliac globulin, no constant stigmata of carriers were demonstrated by Merskey and Macfarlane (1951). Negative results were also obtained by Graham (1956), both in human and in canine hemophilia. Thus Andreassen's claim that the mothers of five isolated cases were carriers cannot be substantiated.

SEGREGATION ANALYSIS OF ISOLATED AND FAMILIAL CASES

Haldane made some rough calculations of the frequency of sporadic cases from the pedigree of Andreassen (1943) in Denmark, Birch (1937) in Illinois, and Hoogvliet (1942) in Holland. Since then, Fonio (1954) has published his summary of hemophilia in Switzerland which Vogel (1955) considered to support Haldane's argument. Their estimates were neither efficient nor unbiased, and no attempt was made to test the significance of the alleged shortage of sporadic cases.

Morton (1958, 1959) and Morton and Chung (1959) provided a maximum likelihood solution to the problem of estimating the frequency of sporadic cases in sex-linked inheritance. Information is provided primarily by index sibships (with at least one proband) and by their maternal uncles. A small amount of information furnished by more remote relatives will be ignored, since it is difficult to ascertain reliably without unconscious selection for positive family histories. The relevant probabilities are:

for an index sibship of s brothers to have r affected (Morton, 1959)

$$P(r = 1/r > 0) = \frac{sp\pi[x + (1 - x)\, q^{s-1}]}{xsp\pi + (1 - x)\,[1 - (1 - p\pi)^s]}$$

and

$$^{p}(r/r > 1) = \frac{\binom{s}{r} p^r q^{s-r}\,[1 - (1 - \pi)^r]}{1 - (1 - p\pi)^s - \pi spq^{s-1}},$$

for at least one of s maternal uncles to be affected if there is a single affected nephew in an index sibship of n nephews (Morton and Chung, 1959)

$$P = \frac{(1 - x)(1 - x^1)\, q^{n-1}\, (1 - q^s)}{x + (1 - x)\, q^{n-1}},$$

for at least one of s maternal uncles to be affected if there are two or more affected nephews in an index sibship of n nephews (Morton and Chung, 1959)

$$P = (1 - x^1)(1 - q^s),$$

for an index sibship with r affected to have *a* probands (Morton, 1959)

$$P = \frac{\binom{r}{a} \pi^a\, (1 - \pi)^{r-a}}{1 - (1 - \pi)^r},$$

where

$p = 1/2$ is the segregation frequency from carrier mothers and and $q = 1 - p = 1/2$

x is the proportion of cases in the population that are sporadic mutants from noncarrier mothers

π is the probability that a case in the population be a proband

$x^I = 1/2$ is the probability that the mother of a carrier female not be a carrier, and therefore the carrier's brothers not be at risk.

Maximum likelihood scores for these parameters provide a test of the null hypothesis that $p = 1/2$, $x = m/3$, and $x^I = 1/2$.

The only practical difficulty in applying this analysis to the literature is that the method of ascertainment was unspecified and likely to have been irregular, with selection of large pedigrees. This bias is minimized in the four systematic studies of Andreassen, Birch, Fonio, and Hoogvliet, but information about ascertainment is poor. Andreassen never indicated more than one proband per kindred, yet he ascertained his material from the Invalidity Insurance Council, the hospitals of Denmark, and the Danish general practitioners. He believed that he had ascertained all cases living in Denmark, and it seems likely that he approximated this objective, even if he did not reach it. Therefore, the absence of multiple probands can only be due to failure to record probands after the first in each kindred. This leads to an underrepresentation of index families in large pedigrees, which would tend to overestimate the proportion of sporadic cases. Fortunately, he indicates which of his cases were examined in

hospitals. Since these patients would have been probands if the ascertainment were through hospital records, sibships containing such patients have been taken as index sibships. Hoogvliet did not indicate his probands, and we have taken the patients that were examined for both hemophilia and color blindness as probands. Birch considered all patients at the University of Illinois Hospital to be probands. Fonio did not designate probands, and we have included all sibships with living cases as index sibships, assuming that, like Andreassen, he had ascertained most of the cases living in his country. Since it is likely that some of the cases considered to be probands by these definitions were in fact secondary cases which were ascertained and examined because of an affected relative, the number of index sibships in large pedigrees will tend to be overestimated, and the sporadic cases to be underestimated.

Because of the inadequacy of the ascertainment data, it is impossible to estimate π precisely. An upper limit may be obtained on the assumption that all living cases were ascertained. Andreassen reported that the mean life of a hemophiliac between 1860 and 1925 was 18 years, while that of Danish males in 1905 was 55 years. Therefore, in the set of cohorts which make up the population, only $18/55 = .327$ of the hemophiliacs who would be present if their survival were normal are now alive, and this is the maximum ascertainment probability (π) if all living cases were ascertained. A more exact calculation is based on the cumulative survival of hemophiliacs by age, $H(x)$, the survival in the general population, $G(x)$, and the distribution of age, $f(x)$, in the general population. This gives $(\pi) = \int H(x) f(x)/G(x) = .319$ as the maximum ascertainment probability, computed from Andreassen's mortality data and the national age and mortality distributions.

However, this maximum ascertainment is unlikely to have been realized, even though the studies were conducted over several years and had a chance to ascertain some cases that died before the end of the investigation. Many cases dying at birth or during the first week of life would be missed, as would living cases who had not yet had severe bleeding. These losses would be especially likely for sporadic cases. Andreassen reported eighty-one living cases in Denmark in 1943, and Fonio found seventy-nine living cases in Switzerland. However, Hoogvliet reported only seventy-five living cases in Holland, and Birch fewer than one hundred cases in Illinois, each with a population about twice as large as Denmark or Switzerland. It is not stated that all of Birch's patients were alive and resident in Illinois. Even if Andreassen's and Fonio's studies were nearly complete, with an ascertainment probability approaching .30, the studies of Birch and Hoogvliet could not have attained an ascertainment probability more than half of this, and quite likely less.

Accordingly, we first analyzed the data according to single selection ($\pi \rightarrow 0$), for which it is appropriate to deduct one patient from each index sibship and then analyze the sibs as if they were obtained under complete selection (table 1). There is a striking contrast between Birch's study and the others, which show no significant deviation from the assumed parameters ($p = 1/2$, $x = 1/4$). Birch's data, however, show a marked deficiency of sporadic cases, coupled with a suspiciously high segregation frequency, which is nearly significant even when attention is restricted to multiplex families ($\chi^2 = 3.74$).

Even when p is fitted simultaneously, the deficiency of sporadic cases in Birch's data remains significant ($\chi^2 = 5.34$), and the heterogeneity with the other studies approaches significance ($\chi^2 = 3.71$). For Birch's study, $x = .0445 \pm .0889$, while for the others, $x = .2517 \pm .0676$. Pooling these two estimates, we obtain $.1758 \pm .0538$, which is still in good agreement with $x = .25$, and therefore, with the assumption of equal mutation rates in egg and sperm. On the basis of the analysis under single selection, we have no reason to reject this hypothesis.

It seems worthwhile also to examine the data using the maximum ascertainment probability, $\pi = .30$, to determine whether our conclusion depends on the ascertainment model (table 2). In Fonio's data the scores for the distribution of living cases among affected are highly discrepant ($U_\pi = 42.9$ $K_\pi = 105.3$, $\chi^2 = 17.51$), with probable heterogeneity among families ($\chi^2_{23} = 34.53$, $p \cong .05$). This is because sibships with living cases are a selected group, in which mortality is less than in the whole population of hemophiliacs. Therefore the distribution of living cases in Fonio's material cannot be used to estimate the ascertainment probability, and has been omitted from table 2.

The situation is little better in the other studies. The total score for π is not significantly discrepant from $\pi = .30$, but there is gross heterogeneity among families. For Birch, $\chi^2_{31} = 61.95$; $P < .001$; for Andreassen, $\chi^2_{12} = 22.26$, $P = .04$. This is probably due to inclusion of secondary cases as probands, with variable likelihood among families. The estimate of π cannot be trusted and is very likely too high, while the estimate of sporadic cases is too low, both through inclusion of nonindex sibships and because of the negative covariance of the estimates of x and π.

Setting aside natural reservations, we find that the studies excluding Birch are still in good agreement with the null hypothesis ($x = .2144 \pm .0665$). However, the deficiency of sporadic cases and segregation disturbance in Birch's material are even more marked than before. In multiplex families, $p = .694 \pm .077$. Fitting p simultaneously, $x = .0174 \pm .0870$. Since there are discrepancies in both parameters, it seems necessary to provide an explanation. With regard to the segregation frequency p, it seems important that Birch obtained most of her pedigree information "from facts recalled by the patients themselves, their mothers, and other members of their families. . . Where the chief source of information is memory, there are bound to be errors" (Birch, 1937, pp. 7-8). She lists 267 hemophiliacs and 33 "questionable hemophiliacs" in the pedigrees. Some of the latter occurred in the index families and were considered by us to be normal. They include individuals whose medical history was "died at three weeks, of convulsions" (chart 21, III-1) and "died on the second day after his birth, cause unknown" (chart 18, IV-3). Moreover, some of the males listed by Birch as affected were doubtfully so. For example, II-12 in chart 21 "is said to have been a bleeder," and II-3 in chart 31 "is said to have had hemophilia. He died at the age of nine months from pneumonia." Birch reported that 40 of 115 dead hemophiliacs died during the first year of life, whereas Andreassen found only 3 cases in 105 deaths. If Birch was right, then many sporadic cases must have been missed in other studies. However, it may be wondered whether all the males thought by Birch to have hemophilia,

TABLE 1. SEGREGATION ANALYSIS FOR SINGLE SELECTION
($p = \frac{1}{2}$, $x = \frac{1}{4}$)

Source	U_p	U_x	U_{x^1}	K_{pp}	K_{xx}	$K_{x^1x^1}$	K_{px}	K_{xx^1}	$\frac{U_p^2}{K_{pp}}$	$\frac{U_x^2}{K_{xx}}$	$\frac{U_{x^1}^2}{K_{x^1x^1}}$
Andreassen	1.151	12.093	11.470	143.53	92.71	58.98	-45.71	26.95	.009	1.577	2.231
Birch	41.501	-39.689	-21.345	287.59	157.80	137.90	-94.95	44.86	5.989	9.982	3.304
Hoogvliet	13.029	-1.251	8.976	69.28	32.23	21.83	-20.10	2.45	2.450	.049	3.691
Fonio	-14.136	-10.482	-5.054	212.55	151.04	112.69	-90.06	47.21	.940	.727	.227
Total	41.545	-39.329	-5.953	712.95	433.78	331.40	-250.82	121.47	2.421	3.566	.107
Total (excluding Birch)	0.044	0.360	15.392	425.36	275.98	193.50	-155.87	76.61	.000	.000	1.224

Analysis of χ^2

	d.f.	χ^2	p
p, Birch vs. others	1	3.57	.1-.05
p, among others	2	3.40	.2-.1
x, Birch vs. others	1	6.42	.02-.01
x, among others	2	2.35	.5-.3

Birch, fitting x and p simultaneously: $x = .0445 \pm .0889$

Others, fitting x and p simultaneously: $x = .2517 \pm .0676$

Pooled $x = .1758 \pm .0538$

Heterogeneity, $\chi^2_i = 3.709$

TABLE 2. SEGREGATION ANALYSIS FOR MULTIPLE SELECTION
($P = \frac{1}{2}$; $x = \frac{1}{4}$, $\pi = .30$)

Source	U_p	U_x	U_π	K_{pp}	K_{xx}	$K_{\pi\pi}$	K_{px}	$K_{p\pi}$	$K_{x\pi}$	$\frac{U_p^2}{K_{pp}}$	$\frac{U_x^2}{K_{xx}}$	$\frac{U_\pi^2}{K_{\pi\pi}}$
Andreassen	7.886	6.981	2.176	136.57	97.61	81.44	-41.19	-20.90	17.96	0.455	0.499	0.058
Birch	53.27	-48.314	-1.813	259.60	163.73	189.77	-88.80	-42.53	30.41	10.931	14.257	0.017
Hoogvliet	15.994	-3.489	-2.432	63.43	34.39	2.59	-18.24	-9.91	7.87	4.033	0.354	2.284
Fonio	-3.756	-17.987	-1.159	210.26	155.17	8.29	-85.26	-36.22	26.48	0.067	2.085	0.162
Total	73.394	-62.809	-3.228	669.86	450.90	282.09	-233.49	-109.56	82.72	8.042	8.749	0.037
Total (excluding Birch)	20.124	-14.495	-1.415	410.26	287.17	92.32	-144.69	-67.03	52.31	0.987	0.732	0.022

	d.f.	χ^2	p
p Birch vs. others	1	3.876	.05-.02
p among others	2	3.568	.2- .1
x Birch vs. others	1	6.240	.02-.01
x among others	2	2.206	.5- .3

Birch, fitting x, p, π simultaneously: $x = .0174 \pm .0870$

Others, fitting x, p, π simultaneously: $x = .2144 \pm .0665$

Mean x $x = .1418 \pm .0529$

Heterogeneity, $\chi^2_i = 3{,}236$

especially the infant deaths not certified by a physician, were in fact affected. It seems likely that the excess of affected males in these families is an artifact, since it is still apparent even on the assumption of single selection.

Birch laid unusual stress on a positive family history in the diagnosis of hemophilia. "Two findings should be essential to the diagnosis of hemophilia: (1) a history of habitual bleeding, both spontaneous and following slight injury: and (2) prolongation of the coagulation time. If sufficient family history is obtainable, there should also be evidence that the disease is hereditary." (Birch, 1937, p. 40). "Certainly, in the vast majority of cases, the disease is hereditary, although sometimes difficult to trace" (p. 66). This emphasis on a positive family history may well explain not only the apparent overdiagnosis of hemophilia in these pedigrees, indicated by the segregation disturbance, but also the deficiency of sporadic cases. There was more opportunity for such unconscious bias in Birch's material, since the smaller incidence of living cases indicates a less complete ascertainment than in the studies of Andreassen and Fonio.

Although we believe that the evidence is sufficient to set aside Birch's data in favor of the other studies which reveal no deficiency of sporadic cases, there is no significant evidence of such a deficiency even when all the data are pooled, giving $x = .1418 \pm .0529$, with an upper 95 per cent confidence limit of $.1418 + 1.645(.0529) = .229$. We have seen that estimates of the selection coefficient m range from .75 to .43, so that the expected value of x with an equal sex ratio of mutation rates lies between .25 and .15. Vogel (1955) calculates $m = .62$ from Fonio's data, which would give $x = .207$ as the expected proportion of sporadic cases. Even though the proportion of sporadic cases is underestimated by the assumption of a maximum value of π, with no selection of hereditary cases, the estimate is still not significantly less than the value expected with equal mutation rates in egg and sperm.

DISCUSSION

Although the data on hemophilia do not demonstrate a deficiency of sporadic cases, they do not rule out this possibility. Taking the pooled data for single selection, a 95 per cent confidence interval for x is $.1758 \pm 1.96$ (.0538), or .070 to .281. With $m = .62$, this corresponds to a confidence interval for the sex ratio of the mutation rates (v/u) of 6.86 to .21. Thus the rate in sperm could be six times the rate in eggs or vice versa. The real range is even greater than this, since the selection coefficient m is not known with certainty.

For Duchenne muscular dystrophy the limits are appreciably narrower, because the selection coefficient is nearly unity (Morton and Chung, 1959). The estimate for x is $.355 \pm .050$, giving a 95 per cent confidence interval of .257 to .453. The corresponding interval for v/u is 1.89 to .21, so that the mutation rate in sperm is unlikely to be as much as twice as great as in eggs.

This conclusion is subject to several reservations. Phenocopies may add to the sporadic cases, although none has ever been demonstrated for hemophilia. There is a sporadic limb-girdle type of muscular dystrophy which sometimes

mimics the sex-linked type, but its frequency among all limb-girdle cases is nearly the same as the expected frequency of sporadics in sex linkage, so that no appreciable bias could be caused by admixture from this source.

Somatic mutations seem unlikely to be an important cause of sporadic cases, since 10 per cent of normal serum will correct the clotting defect in the serum of hemophiliacs (Merskey and MacFarlane, 1951), and heterozygous women are normal. Therefore mutations in a clone of the cells responsible for AHG production should be clinically undetectable. Similarly, it is doubtful that a somatic mutation in a clone of muscle cells could counterfeit Duchenne muscular dystrophy, given that the heterozygous carrier is usually normal. Autosomal coagulation defects and muscular dystrophies are known, but the differential diagnosis usually is not difficult and in any case they would affect p more than x. There may be more than one complex locus for Duchenne dystrophy and there is certainly more than one locus represented in the older studies on hemophilia, which did not differentiate Christmas disease. This however would not affect the estimation of x or its interpretation in terms of mutation rates, except that v and u now represent the sum of several rates. Despite the defects in the available data, they do not seem to be of a kind to cast doubt on the conclusion that mutation rates are similar in eggs and sperm.

Evidence from lower organisms concerning the relative frequency of spontaneous mutation in the two sexes is inconclusive. Drosophila has given contradictory results (Glass and Ritterhof, 1956; Muller, Valencia, and Valencia, 1950). Whatever the true situation in Drosophila may be, we cannot extrapolate to mammals because of the difference in oogenesis, which continues through the lifespan of a female Drosophila but is believed to stop shortly after birth in mammals (Parkes, 1956). Spontaneous mutation rates in both sexes have been measured by Russell *et al.* (1959) and Carter (1958) in mice. They found no mutations at seven loci among 46,763 eggs as compared with twenty-one mutants among 329,878 sperm. However, this difference is not significant (χ^2 with Yates' correction = 1.945, p. = .16).

It is interesting to trace the consequences of the assumption that mutation rates are nearly equal in the two sexes. We know that mutations can take place in nondividing cells, like sperm (Muller, 1946), and that they increase in frequency with paternal age but not appreciably with maternal age (Muller, 1946; Penrose, 1955; Morton and Chung, 1959). Gonial mutations are rarely detected in mammals (Reed and Falls, 1955), and in Drosophila the postgonial stages beginning with meiosis and ending with early cleavage of the zygote are inordinately mutable (Muller, 1946, 1959; Hildreth and Carson, 1957). These facts can be economically related to equal mutation rates in the two sexes if we assume that a minority of mutations take place in gonial cells, at rates proportional to the number of cell divisions, but the majority arise during gametogenesis, the haplophase, and early cleavage, at rates independent of whether the homologue is an X or Y chromosome. From the first assumption it follows that mutations increase with paternal age but not with maternal age, since spermatogonia divide constantly throughout adult life, while oogonia apparently stop dividing soon after birth. Alternatively, prolongation of the haplophase due to lessened sexual activity may account for the paternal age

effect. From the second assumption it follows that mutation rates are nevertheless nearly equal in egg and sperm, and that studies of the effect of the age of the maternal grandfather on sex ratio of grandchildren can detect only a minority of sex-linked lethals (Cavalli-Sforza, 1962). However, this hypothesis is only tentative, and there may be intrinsic mutability differences between spermatogonia and oogonia which counterbalance the difference in number of cell divisions and thus lead to nearly equal mutation rates in eggs and sperm even if most mutations are gonial.

SUMMARY

The proportion of cases of a sex-linked trait that are sporadic depends on the relative mutation rates in egg and sperm, and this was the basis of Haldane's suggestion that mutations are much more frequent in sperm than in eggs. We have reexamined this question by segregation analysis of several studies on hemophilia. The data do not meet present standards of regular ascertainment and differential diagnosis; the amount of information is relatively small, and a deficiency of sporadic cases cannot be excluded. However, there is no evidence of a deficiency of sporadic cases in these samples. Larger genetic surveys, conforming to regular ascertainment models, are needed to detect a deficiency of sporadic cases if it exists. Meanwhile, the contention that studies of sex-linked mutants in man reveal a deficiency of sporadic cases which could be due to a higher mutation rate in sperm than in eggs, is not supported. Substantially equal mutation rates, in conjunction with an effect of paternal age but not of maternal age, would suggest that postgonial stages in mammals, as in Drosophila, are extremely mutable and account for a large fraction of spontaneous mutations.

REFERENCES

ANDREASSEN, M. 1943. Haemofili i Danmark. *Opera ex Domo Biol. Hered. Hum. Univ. Hafniensis* 6: 1-168.

BENZER, S. 1957. The elementary units of heredity, in *The chemical basis of heredity*, W. D. McElroy and B. Glass, ed. Baltimore: Johns Hopkins Press, pp. 70-93.

BIRCH, C. L. 1937. Hemophilia, clinical and genetic aspects. *Illinois Mono. Med. Sci.* 1: 1-151.

CARTER, T. C. 1958. Radiation induced gene mutation in adult female and foetal male mice. *Brit. J. Rad.* 31: 407-411.

CAVALLI-SFORZA, L. 1962. A. method for estimating mutation rates in man. (In preparation.)

CHEESEMAN, E. A., KILPATRICK, S. J., AND STEVENSON, A. C. 1958. The sex ratio of mutation rates of sex-linked recessive genes in man with particular reference to Duchenne type muscular dystrophy. *Ann. Hum. Genet.* 22: 235-243.

FONIO, A. 1954. Die erblichen und die sporadischen Blutterstämme der Schweiz. *Bull. Schweiz. Akad. Med. Wiss.* 10, (5), 123 pp.

GLASS, B. AND RITTERHOFF, R. K. 1956. Spontaneous mutation rates at specific loci in Drosophila males and females. *Science* 124: 314-315.

GRAHAM, J. B. 1956. Biochemical genetics of blood coagulation. *Amer. J. Hum. Genet.* 8: 63-79.

HALDANE, J. B. S. 1935. Rate of spontaneous mutation of a human gene. *J. Genet.* 31: 317-326.

HALDANE, J. B. S. 1947. The mutation rate of the gene for hemophilia and its segregation ratios in males and females. *Ann. Eugen.* 13: 262-271.

HALDANE, J. B. S. 1956. Mutation in the sex-linked recessive type of muscular dystrophy—possible sex-difference. *Ann. Hum. Genet.* 20: 344-347.

HILDRETH, P. E., AND CARSON, G. L. 1957. Influence of the type of inseminated female on the lethal frequency in the X chromosome from the male. *Proc. Nat. Acad. Sci.* (Wash) 43: 175-183.

HOOGVLIET, B. 1942. Genetische ci Klinische Beschonivingen Naar Aanleiding Van Bloedersziekte en Kleurenblindheid in Dezelfde Familie. *Genetica* 23: 93-220.

MERSKEY C. AND MACFARLANE, R. G. 1951. The female carrier of hemophilia: A clinical and laboratory study. *Lancet* 1: 487-490.

MORTON, N. E. 1958. Segregation analysis in human genetics. *Science* 127: 79-80.

MORTON, N. E. 1959. Genetic tests under incomplete ascertainment. *Amer. J. Hum. Genet.* 11: 1-16.

MORTON, N. E. AND CHUNG, C. S. 1959. Formal genetics of muscular dystrophy. *Amer. J. Hum. Genet.* 11: 360-379.

MULLER, H. J. 1946. Age in relation to the frequency of spontaneous mutations in Drosophila. *Yr. Bk. Amer. Philos. Soc* 1945: 150-153.

MULLER, H. J., VALENCIA, J. I., AND VALENCIA, R. M. 1950. The frequency of spontaneous mutation at individual loci in Drosophila. *Genetics* 35: 125-126.

MULLER, H. J., 1959. Advances in radiation mutagenesis through studies on Drosophila. Progress in nuclear energy, series VI(2) *Biol. Sc.* 2: 146-160.

PARKES, A. S. 1956. *Marshall's physiology of reproduction.* New York: Longmans, vol. 1, pp. 425-427.

PENROSE, L. S. 1955. Parental age and mutation. *Lancet* 2: 312-313.

REED, T. E., AND FALLS, H. F. 1955. A pedigree of aniridia with a discussion of germinal mosaicism in man. *Amer. J. Hum. Genet.* 7: 28-38.

ROBERTS, J. A. F. 1959. *An introduction to medical genetics.* London: Oxford University Press, p. 97.

SMITH C. A. B., AND KILPATRICK, S. J. 1958. Estimates of the sex ratio of mutation rates in sex-linked conditions by the method of maximum likelihood. *Ann. Hum. Genet.* 22: 244-249.

STERN, C. 1960. *Principles of human genetics* (2d ed.) San Francisco; W. H. Freeman and Co., p. 461.

VOGEL, V. F. 1955. Vergleichende Betrachtungen über die Mutationsrate der geschlechtsgebunden rezessive Hamophilieformen in der Schweiz und in Danemark. *Blut* 1: 91-109.