Among the species grown by Burkholder and Sinnott was a strain of *Sordaria fimicola* Ces. and De Not. We wish to report the formation of mature and apparently normal perithecia by a strain of this species when grown under "deep vat" conditions. Our observation would seem to open the possibility of undertaking an experimental analysis of the intrinsic and extrinsic factors which control the onset of fruiting and the production of ascospores. Submerged cultures, as shown by Burkholder and Sinnott, take the form of small, discrete mycelia, which can be counted, transferred intact to Warburg respirometers, and otherwise manipulated as units for the purposes of experimentation.

The medium used was essentially that of Westergaard and Mitchel (*2*), without agar, and modified to contain 8–10 μg of biotin per liter and 5% sucrose. The pH range before inoculation was 4.0 to 4.5, and the temperature of incubation was about 25° C. Inoculations were made by adding an aliquot of an ascospore suspension prepared in a loosely fitting Potter homogenizer as suggested by Burkholder (*3*). The culture vessels were tubes measuring approximately 50 × 400 mm. Some of the tubes had an air inlet to the bottom ring-sealed into the top and a side-arm air outlet also sealed to the tubes; others were provided with rubber stoppers carrying an air inlet and outlet. Air, sterilized by passing through cotton, and humidified by passing through sterile water, was passed into the cultures at a rate that caused constant vigorous bubbling.

After about 4 days, the medium began to darken somewhat and to foam in a way characteristic of protein solutions, and soon thereafter the fungus apparently began to grow more rapidly and the mycelium took on a light-brown color. After about 2 weeks, young to fairly mature perithecia were formed on the newer part of the mycelium. Eventually some of these matured, and the ascospores were shed into the medium. At the time of fruiting, the cultures were very thick and dark, and the mycelium was a tan color under the microscope. It should be noted, however, that the individual colonies here were of a much looser texture than the colonies of this fungus studied by Burkholder and Sinnott, and also that they were not perfectly regular in shape. The largest colonies were about 1 cm in diameter, and had larger, more mature, and greater numbers of perithecia than did the smaller colonies.

On lower concentrations of sucrose and on unfavorable carbon sources such as inulin, young perithecia can be seen on the very scanty mycelium in 4–6 days, and very little further mycelial growth occurs.

Apparently strictly aerobic conditions are not necessary for the formation of the perithecia of this fungus. This was also noted by Edwards *et al.* (*4*) for this species and by Denny (*5*) for *Neurospora crassa*.

We have also noted the formation of submerged perithecia of *Sordaria* in standing liquid cultures containing low concentrations (less than 1%) of sucrose and other carbon sources. Formation of immature perithecia of *N. crassa* (15,300 A × 15,300 a) occurred in standing liquid cultures containing the nonnitrogenous mineral constituents of the Westergaard and Mitchel medium and 0.2% Difco yeast extract but lacking sugar. These perithecia failed to mature.

The ascospores of this strain of *S. fimicola*, which normally seem to germinate only to the extent of about 5% in sugar-containing media, may be germinated to advantage in the above-mentioned culture vessels, using 0.5–1.0% sodium acetate instead of sucrose in the Westergaard and Mitchel medium. At least 60% germination may be secured in 18 hr at 25° C; after this length of time, further counts are not feasible.

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The Rate of Mutation of the Gene Responsible for Retinoblastoma in Man

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Retinoblastoma is a highly malignant ocular neoplasm that characteristically appears in children ranging in age from a few months to four years. Unless effective surgical or irradiation therapy is initiated before the lesion has spread beyond the confines of the globe of the eye, the outcome is almost invariably fatal. Most children with the disease have no similarly affected relatives. The literature contains numerous reports, however, of families in which individuals surviving the disease have transmitted it to one or more of their children. There are also reports of several affected children being born to normal parents (*1–4*).

Current genetic theory (*3, 5, 6*) holds that the disease is due to a dominant gene. The apparently isolated cases are attributed to mutation. In a state of nature the genes responsible for retinoblastoma would for the most part tend to be eliminated by natural selection in the same generation that they arose through mutation. When, however, either because of appropriate medical treatment or in consequence of a very rare spontaneous regression of the tumor, an affected individual survives, he may transmit the gene to half his children, who in turn may develop the disease. It is further postulated that occasionally, for unknown reasons, an individual who possesses this gene fails to develop a retinoblastoma. He will still transmit the

[1] This study has been supported by a grant from the American Cancer Society, to which grateful acknowledgment is made. A detailed report on this investigation will be published elsewhere (*Arch. Ophthalmol.* [Chicago], in press).

gene to half his children, however, and they will develop the disease unless the same or some other suppressive influence is operative.

If one assumes that all sibships in which one or more children with retinoblastoma are born to normal parents are due to a dominant mutation involving the same genetic locus, then it is possible to estimate the rate with which this mutation is occurring. The fact that the gene is not completely penetrant does not significantly alter the estimate. Each gene that arises in one generation but fails to find expression will be approximately balanced by a gene that arose in a previous generation but is only now finding expression in the children of the person who first received it. The estimate of mutation rate depends upon the derivation of an accurate figure for the frequency of sibships in which one or more affected children have been born to normal parents.

We have now completed a survey of cases of retinoblastoma occurring in the state of Michigan between 1938 and 1947. Individuals with the disease have been located through the records of the Department of Pathology and the University Hospital of the University of Michigan, through correspondence with all practicing ophthalmologists and hospitals in the state, and through the records of the State Department of Vital Statistics and the Michigan State School for the Blind. The diagnosis has in each case been verified through appropriate field studies and professional correspondence. A total of 53 cases located among 52 families is known to have occurred during this period. Since, however, the one instance of two cases in a single family involved identical twins, we are dealing, biologically speaking, with a total of only 52 cases. In two instances the affected child had an affected parent. In one instance, although both parents were normal, the affected child had 5 affected siblings, all born outside the 10-year period under investigation. The mutation rate may be derived from the ratio

$$\frac{\text{Number of sibships in which one or more affected children have been born to normal parents during 1938–47}}{\text{Total number of births in the population during 1936–45}} = \frac{49}{1{,}054{,}985}.$$

The reason for using the number of births during 1936–45 rather than 1938–47 is that on the average children with the disease do not come to diagnosis until approximately 2 years of age. Although actually there are 50 sibships that could be included in the numerator of this fraction, the sibship with the 6 affected children, only one of whom was diagnosed between 1938 and 1947, has been excluded because five sixths of the affected individuals were diagnosed outside the period under study. The frequency of critical sibships is thus 0.0000464, with a 19 in 20 probability that the true value lies between 0.0000344 and 0.0000615, as calculated by the method of Stevens (*7*). The actual mutation rate per gene, since each individual is diploid, is 0.0000232, or 2.3×10^{-5}, with a 19 in 20 probability that the true value lies between 1.7 and 3.1×10^{-5}. This estimate appears to be significantly higher than a widely quoted unpublished estimate of Philip and Sorsby (*8*) for the same gene (1.4×10^{-5}), their estimate being based upon material drawn from London. The basis for the difference in the two estimates lies in the lower incidence figure that they adopted. The possibility therefore arises that the rate of mutation of this gene varies significantly from one geographical area to another.

If some cases of retinoblastoma are nongenetic in origin, or if the disease can be caused by the presence of any one of several different genes, then the estimate given above is too high. It has been pointed out that the frequency of bilateral disease is greater where two or more cases appear in a single family than where the disease occurs as an isolated event. In other words, the *proved* genetic disease is more often bilateral than where a genetic origin is presumptive. This raises the possibility that a certain fraction of the isolated cases are due to somatic rather than germinal mutation, in which case the estimate derived above is again too high. If, on the other hand, ascertainment of the trait falls short of 100%—as seems probable—then the estimate is too low. Moreover, the present estimate fails to correct for infant mortality prior to the age of 2, or identical twin births, facts which also tend to result in a lowering of the estimate.

The data have been analyzed for the existence of factors capable of influencing the rate of mutation of this gene. There does not appear to be a significant tendency for a disproportionate frequency of occurrence of retinoblastoma during any of the 10 years under study. Month of conception cannot be shown to exert an effect on the mutation rate. No effect of parental age can be demonstrated. There is no significant difference in the frequency of mutation among urban as compared to rural populations. Family studies reveal no distinguishing physical or mental characteristics of the parents of children with retinoblastoma.

Tentative mutation rate estimates are now available for approximately a dozen human genes. These are summarized in Table 1. The values given are of very unequal degrees of validity (a critical evaluation of the worth of these estimates will be presented elsewhere). In addition to the results presented in Table 1, studies on sickle cell anemia and thalassemia (Cooley's anemia) have led to the postulate that the incompletely recessive genes which produce these two diseases either have in certain racial groups a very high mutation rate, of the order of $4\text{–}10 \times 10^{-4}$, or that a state of balanced polymorphism exists, wherein the negative selection to which the homozygote is subject is offset by a slight positive selection in favor of the heterozygote (*16*). The mutation rate for retinoblastoma here reported falls well within the range of the other values thus far determined.

Although dominant genes are most suitable for mutation rate studies, recessives may also be used, and

TABLE 1

SUMMARY OF ESTIMATES OF THE RATE OF MUTATION OF HUMAN GENES

Classification of gene	Character produced by gene	Mutation rate/ gene/generation	Author
Autosomal dominant	Epiloia	$.8–1.2 \times 10^{-5}$	Penrose, 1936 (*9*)
	Chondrodystrophy	$4.2–4.8 \times 10^{-5}$	Mørch, 1941 (*10*)
	Pelger's nuclear anomaly	8×10^{-5}	Pätau and Nachtsheim, 1946 (*11*)
	Aniridia	$> 1.2 \times 10^{-5}$	Mollenbach, 1947 (*12*)
	Retinoblastoma	1.4×10^{-5}	Philip and Sorsby, unpublished (*8*)
		2.3×10^{-5}	Neel and Falls, this paper
Autosomal recessive	Microphthalmos and anophthalmos with or without oligophrenia	$1–2 \times 10^{-5}$	Sjögren and Larsson, 1949 (*13*)
	Albinism	2.8×10^{-5}	Neel, Kodani, Brewer, and Anderson, 1949 (*14*)
	Congenital total color blindness	2.8×10^{-5}	" "
	Infantile amaurotic idiocy	1.1×10^{-5}	" "
	Ichthyosis congenita	1.1×10^{-5}	" "
Sex-linked recessive	Hemophilia	3.2×10^{-5}	Haldane, 1947 (*15*)

a number have been incorporated into Table 1. The chief pitfalls inherent in attempting to calculate the rate of mutation of nominally recessive genes have been stated by Haldane (*17*) and Muller (*18*). The theoretical objections are chiefly three: lack of information concerning the selective value of the heterozygote, lack of information concerning the mean coefficient of inbreeding that obtained during the centuries when the present-day gene frequency was being established, and insufficient data concerning the reproductive behavior of the parents of defective children with a shortened life expectancy. With respect to the first objection, it should be pointed out that, although it is possible that the heterozygote may conceivably be the object of positive selection in certain instances, it is very improbable that this can be invoked as a general rule to explain *all* instances where recessive genes appear to exhibit high mutation rates. With respect to the second objection, it is apparent that the general reduction in the amount of inbreeding in human populations which appears to have occurred in the past several centuries should on the whole result in an underestimate of the mutation rates of recessive genes, so that the derived figures are minimum. Finally, with respect to the third objection, it must be admitted that there are limited data to indicate that under present-day conditions there may exist a type of reproductive overcompensation among the parents of grossly defective children, so that despite an increased death rate among their children the final sibship size may exceed normal (*19–21*). If such a phenomenon has existed for long, it would introduce significant bias into estimates of mutation rates. However, this is almost certainly a phenomenon of recent origin—it is difficult to believe that during the long centuries when the present gene frequencies were being established, when infant mortality rates of 50% were not uncommon, this factor was operative (*21*). All in all, it does not appear that these various objections are sufficient to invalidate attempts to calculate the rate of mutation of recessive genes, as long as the pitfalls are kept in mind and the results interpreted with due caution. It is noteworthy that the figures given in Table 1 for recessive genes are in substantial agreement with those given for dominants.

The average of the estimates of mutation rate in Table 1 is approximately 2.6×10^{-5}. Although this average is perhaps unduly influenced by the relatively high and in some respects not completely satisfactory figure for Pelger's nuclear anomaly, we shall accept it as the best currently available. In terms of life cycles, this is a significantly higher rate than has been reported for any other animal species. The haploid number of independent loci capable of mutation in man may be placed at approximately 20,000 (*22*). To the extent that one can draw conclusions from this very limited sampling of the human genome, these figures would indicate that on the average the total mutation rate ($2.6 \times 10^{-5} \times 2 \times 10^{4}$) is such that each diploid individual possesses one mutant gene not present in either parent.

Several investigators have suggested that the estimates so far accumulated are not based on "typical" genes, the argument being that since only the more rapidly mutating genes supply sufficient material for mutation rate estimates, there has automatically been an unconscious selection of such genes in the studies thus far carried out (*17, 23*). This suggestion appears to the authors to have only a limited validity. Genes with clear-cut and striking effects, especially dominants, probably tend to have lower mutation rates than those whose effects are less dramatic—this in response to selection pressures. Studies on induced mutation suggest that clear-cut dominant and recessive mutations are distinctly in the minority when one considers the entire spectrum of induced genetic change (*24*). It follows, then, that the mutation rate studies thus far carried out have probably involved the *most* rapidly mutating among a group of genes which as a whole are *less* rapidly mutating than genes whose effects are more subtle. It would therefore appear to be premature to conclude that the rates thus far estimated are higher or lower than the *average*.

Muller (*18*), in a brilliant treatment of the mutation problem in man, has emphasized the relative frequency with which this phenomenon occurs, a point of view with which we are in complete agreement. Our own figure for total mutation rate is somewhat higher than that arrived at by Muller (0.1–0.5), the chief basis for the difference being the more conservative estimate of gene number (5,000–20,000) which he adopted. He has stated that it is unlikely that the total mutation rate in man exceeds 1.0, because, if we assume an approximate equilibrium between the origin of new traits through mutation and their removal through selection, this implies an average of one "genetic death" per individual; it seems to him unlikely that the species could "tolerate" more than this. This concept of "genetic death" is, however, a statistical abstraction that can be misleading. All of us fall far short of the theoretically perfect representative of the species. The various members of a species can each carry a considerable handicap as long as the species as a whole is capable of successfully resisting efforts to dislodge it from its particular ecological niche by other (genetically handicapped) species. Man with his highly developed nervous system and social organization may have developed mechanisms for compensating for theoretical genetic death, mechanisms not operative in lower forms. In other words, the tolerable limit of genetic inefficiency depends upon both inter- and intraspecific selective pressures. Man may have so far negated the interspecific competitions, and so far mitigated and altered the usual intraspecific competitions, that relatively high mutation rates per generation can be tolerated (and on occasion turned to advantage) as long as the integrity of the organ responsible for his success, the brain, is not threatened. Furthermore, the survival of an individual under competition is as a rule not determined by the presence of single genes but by constellations of genes. Each individual who dies for reasons primarily genetic removes some 40,000 genes from circulation. One "genetically determined" death may therefore effect the disappearance of a number of mutations, particularly if there is any tendency for the distribution of unfavorable genes in a population not to follow a normal frequency curve. For these reasons it would seem premature, until more detailed data are available, to postulate a genetically acceptable upper limit for total mutation frequency.

Further research in this area is a prerequisite to intelligent discussion of the problems of induced mutation, as from therapeutic or diagnostic irradiation, or the peacetime or military applications of atomic energy. The dangers of induced mutation can only be evaluated against the background of knowledge of spontaneous human mutation rates. Furthermore, an evaluation of the genetic problems inherent in the recent alterations in the type of selective factors to which human populations are subject likewise revolves around a recognition of the total frequency of mutation which must in each generation in a state of nature be offset by the selective process.

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Cross Resistance of Streptococci to Five *Streptomyces* Antibiotics[1]

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Microorganisms that have acquired resistance *in vitro* or *in vivo* to one antibiotic have been shown to have a cross resistance to other antibiotics to which they had not previously been exposed. This phenomenon is of evident importance in chemotherapy, and may be of significance in understanding the mechanisms of antibiotic activity.

Pansy *et al.* (*1*) induced resistance to chloromycetin and aureomycin separately in strains of *Escherichia coli* and *Micrococcus pyogenes* var. *aureus*. Each resistant strain showed cross resistance to the other antibiotic as well as to terramycin. Herrell *et al.* (*2*) showed that strains of *E. coli* and *Aerobacter aerogenes* resistant to terramycin were also resistant to aureomycin and chloromycetin, but not to streptomycin. *Streptococcus fecalis* and *M. pyogenes* strains resistant to terramycin were resistant to aureomycin, but not to streptomycin or chloromycetin. In contrast, Waksman (*3*) has reported that both streptomycin-sensitive and streptomycin-resistant strains of different mycobacteria were sensitive to neomycin, and

[1] This investigation was supported by research grants from the National Institutes of Health, USPHS, and Abbott Laboratories.

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