

## Genetic Effects

The discoveries of radiation and radioactivity were about as close to Charles Darwin's *Origin of Species* (published in 1859) as we are to America putting a man on the moon. By the time of Röntgen and Becquerel the theory of natural selection was widely accepted but there was still little understanding of the process of inheritance and of the source of the variation needed for natural selection to work on. Darwin's own pangenesis theory of inheritance held some sway. It assumed that each organ produces microscopic “gemmules” that passed around the body and entered the sex cells, each of them carrying the properties of the organ and cells it originated in. As the embryo formed, the gemmules from mother and father were supposed to interact with its new cells to create cells and organs of the appropriate kind and pass on some particular properties from the parents. The theory accounted quite reasonably for many of Darwin's observations, particularly on cross-breeding, but even he thought of it only as a working hypothesis.

It was 1900 before the rules of inheritance began to be widely understood. In one of the more dramatic rediscoveries of science, three scientists independently doing plant breeding experiments found that there were relatively simple rules for the transmission of characteristics between generations. These rules, observed by Hugo de Vries, Carl Correns, and Erik von Tschermak, had actually been discovered (and published) more than 30 years before by the Augustinian monk Gregor Mendel after a meticulous series of experiments on edible peas at his abbey in Brünn (now Brno in the Czech Republic).

Mendel found that the transmission of many characteristics of the peas could be predicted if it were assumed that each pea plant had just two genes (to use the modern term) that determined whether a particular characteristic appeared or not. If there was a gene R that caused round seeds and another r that caused wrinkled seeds then Mendel found the following rules applied:

- Each parent had two genes associated with a particular

characteristic and passed one of these, chosen at random, to each offspring

- If the offspring received an R gene from each parent or one R and one r (i.e. RR or Rr) they would have round seeds; when offspring inherited two r genes (so rr) they would develop wrinkled seeds. The R gene was thus dominant (only one was needed) and the r gene recessive (two were needed if a change was to be seen).

With these simple rules Mendel found that he could predict the statistical outcome of crosses of various types (and with seven different characteristics) with remarkable accuracy.

His results were reported in a lecture to the Natural History Society of Brünn in 1865 and published the following year but by the time the principles were rediscovered in 1900 he had been dead 16 years. His ideas were not immediately accepted even when rediscovered and it was largely through the conviction and energy of the British geneticist William Bateson that, by 1910, Mendelism was accepted as the conceptual basis for inheritance. Although there are, as we shall see, many instances where it is more complex than Mendel thought (even he appears to have developed doubts about its universality), his ideas remain key insights of genetics. Mendel had no idea how his rules were realised; a reasonable understanding of the mechanics behind them was more than a hundred years away.

Even while Mendel remained undiscovered, another strand of genetics was developing. Cells had been seen through the earliest microscopes but, enabled by the developments in microscopes and staining techniques for visualising cell structures, the division of cells in mitosis and the chromosomes (the term was coined by the German Wilhelm Waldeyer to reflect the way they stained) were first seen independently by three biologists in 1873.

Mitosis, the normal way in which cells proliferate, was described in salamander larvae with just about the detail we see in textbooks today by Walther Flemming in 1882 and the process of sexual reproduction began to be understood soon after. The fusing of sperm and ovum (the sex cells) in fertilization of the sea urchin had been seen in 1876 by Oskar Hertwiga and shortly after this several workers, studying *Ascaris*, a parasitic worm with just four chromosomes, realised that the sex cells had half the number of chromosomes (were haploid) of normal (diploid) cells. By 1890 the way in which sex cells were produced in meiosis was broadly understood – although there were important surprises to come.

As early as 1866 Ernst Haeckel had speculated that the nucleus might be the seat of inheritance. But it was not until nearly 20 years later, in 1884,

that four German scientists working independently all suggested that that chromosomes might be the carriers of information. So, by the end of the century it was a reasonable hypothesis that the chromosomes had something to do with inheritance. It was advanced greatly by the work of Theodor Boveri - who showed that a full set of chromosomes was necessary for normal development of sea urchin embryos - but it was Walter S Sutton's work on grasshoppers, published in papers in 1902 and 1903, that brought general acceptance. Sutton showed that the chromosome structures persisted across fertilization and that each chromosome was probably linked to some specific characteristics - they followed the logic of Mendel's genes. Or as Sutton himself put it [153]:

I may finally call attention to the probability that the association of paternal and maternal chromosomes in pairs and their subsequent separation during the reducing division as indicated above may constitute the physical basis of the Mendelian law of heredity. To this subject I hope soon to return in another place.

So by the early years of the twentieth century and within a few years of Röntgen and Becquerel's discoveries, the notional genes of Mendel and the chromosomes seen through a microscope were drawn together. However, it was the second decade of the 20th century before Mendelism and chromosomes were truly reconciled.

In 1910 the American geneticist Thomas Hunt Morgan, working at Columbia University, chose a small fly with a taste for ripe fruit, *Drosophila melanogaster*, as the subject for genetic experiments. This small fly, about 3 mm long, proved a fortunate choice. When a pair was sealed in a small bottle with some simple fruity food they produced several hundred young within just two weeks; experimenting was cheap and the flies were large enough to study with a hand lens.

Linked genes – ones that were located on the same chromosome and were therefore expected to follow one another across generations – were soon encountered in the *Drosophila* work. Sutton had already predicted this but the analysis of the results of crosses showed that the linkage was not invariable – the genes on the same chromosome were not always inherited together - suggesting that there was a transfer of genetic material between chromosomes. The formation of cross-like structures by chromosomes during meiosis had been seen by the French biologist F A Janssens in 1909 (he called them chiasma) and he had speculated that this corresponded to breaking and rejoining (crossing over) of chromosomes with exchange of genetic information. With the *Drosophila* experiments Morgan was soon able

to show that this fitted in with the behaviour seen in linked genes. This was an important step in understanding how nature injected genetic variation through sexual reproduction but it also gave a way to begin mapping the genes because the closer together two linked genes were on a chromosome, the more likely they were to stay linked in offspring. This phenomenon allowed one of Muller's students, Arthur Sturtevant, to begin to map the genes linearly onto the chromosomes.

All this strengthened the hypothesis that chromosomes were the hereditary material but it was Calvin Bridges, another of Morgan's protégés, who published results in 1914 and 1916 showing that, for characteristics known to be sex-linked, the genes were located on the sex chromosomes. This was generally regarded as convincing proof that the chromosomes were the physical embodiment of the genes but the clinching cytogenetic evidence for crossing over (i.e. actually seeing it take place) did not come until 1931 with the work of Barbara McClintock and Harriet Creighton on maize and that of Curt Stern on *Drosophila*.

The term "mutation" was proposed by the Dutch botanist Hugo de Vries between 1901 and 1903 to denote the abrupt changes he saw in the Evening Primrose (*Oenothera biennis*) from generation to generation. While his studies encouraged the idea that mutations could be studied systematically, it was found subsequently that these mutations were not the result of changes in the genes. The Evening Primrose genetics proved difficult to work out but it was eventually clear that the changes were rearrangements of existing genes rather than the creation of new ones. However, de Vries's term stuck and came to mean a heritable change in a gene. Morgan became interested in whether there were mutations in his fruit flies and he soon found one: a male with white eyes, rather than the normal red ones, suddenly appeared. Experiments showed that the white-eye gene followed the X chromosome: more evidence that chromosomes carried the genetic information.

It was the further development of techniques for investigating the occurrence of such spontaneous mutations in *Drosophila* that led to the first demonstration, in 1926, that mutations could arise in offspring because of irradiation of a parent - a true genetic effect.

As we saw earlier, scientists were not slow to study the effects of radiation on living things. While many experiments were unstructured and looked at the effects on the tissues, there were scientists looking at effects at the cellular level and in the cell nucleus. For example, in 1903 Bohn was able to conclude that the main effect of radium treatment on sea urchins was damage to the chromatin. In the following year, after studying the effect of radiation on developing eggs of *Ascaris*, Perthes suggested that the

chromosomes of developing eggs were fragmented. This was confirmed in 1905 by Koernicke who treated *Lilium* (lilies) with radium [154].

So, the fact that radiation could damage chromosomes and therefore disrupt subsequent cell division was known by the end of the first decade of the century. It was clear that rapidly dividing cells were more susceptible (Bergonie and Tribondeau [155]) and it seemed likely that the chromosomal damage was the cause of the somatic radiation effects that were by then known in people. It was Bardeen, in 1906, who was the first to show that irradiated toad sperm led to fertilized eggs that, after a seemingly normal start, failed to develop properly [156]. Since the spermatozoa are entirely nuclear material this showed that it was the nuclear material - the chromosomes - damaged by the radiation that led to the deleterious effects. The results were quickly confirmed with other species such as frogs [157] and rabbits [158].

However, it was the observations of Mavor in the early 1920s that showed convincing evidence of radiation damage to chromosomes. In 1921 and 1922 he showed that doses of x-rays increased the frequency with which chromosomes failed to separate in mitosis and in 1923 [159] he showed that there was also an effect on the frequency of crossing over. This was confirmed by Anderson who, in 1925, also found a female fly with her two X-chromosomes attached to one another after irradiation [154].

Now while this meant that the damage to the chromosomes did affect the individual irradiated (and probably produce the obvious somatic effects) and might persist through a number of normal cell divisions, it did not show a true genetic effect. While the effects of radiation could be seen microscopically on chromosomes as breakages and other gross (in chromosomal terms) damage, there might be other more subtle changes that affected individual genes and could be passed on to offspring. The distinction between chromosomal damage and mutations of individual genes was to be an important, although occasionally fuzzy, one for the rest of the century and the understanding of the ability of radiation to cause mutations made its first major leap forward in 1926 with the work of Hermann J Muller.

Muller was a product of the Columbia University laboratory of T H Morgan and his interest was predominantly in mutations. After his doctoral work at Columbia he took a position at the Rice Institute with the British biologist Julian Huxley. He returned to Columbia in 1918 but his two year appointment was not extended and he left in 1920 for the University of Texas and it was here that he developed an ingenious technique for studying mutations based on a particular strain of *Drosophila* called ClB [160,161]. Using females from this strain Muller was able, through relatively simply

chosen crosses, to detect lethal mutations on the X chromosomes of sperm from normal males: when these were present, after two crosses, there were no male progeny. Simply by counting the number of males it was possible to estimate the mutation rate. The method was sensitive enough to determine the spontaneous mutation rate (about 1 in 1000 for lethal mutations on the X chromosome) and detect a temperature dependence. The temperature dependence arose, he thought, because [162]

...the mutations ordinarily result from submicroscopic accidents, that is, from caprices of thermal agitation, that occur on a molecular and submolecular scale

and this led him to consider that X-radiation might also cause mutations. He used the CIB technique to show quite clearly the effects of irradiating the fly sperm with x-rays: after some 5000 r the mutation rate increased more than 100 times [163]. Writing much later [164], Muller suggested as noteworthy:

...(a) that the induced mutations, like the natural ones, gave evidence of arising in punctiform fashion, in just one or two identical genes in a given cell and (b) that, having arisen, the mutant genes proved to be highly stable and were inherited in the regular Mendelian, chromosomal manner.

In our context the importance of the results was that they showed that radiation could cause mutations - something that was to become a dominant concern of radiation protection years later. At the time, Muller thought it was at least as significant that he had found a means to alter and measure the mutation rate (he saw more mutations in a few months than had been previously seen in all the studies of *Drosophila*) and therefore might have provided geneticists with an important tool to study the genes and inheritance. He was wrong in this; the major advances in genetics were to come through the identification of DNA as the genetic material and the understanding of its structure and chemistry.

Muller just scraped in as the discoverer of radiation-induced mutations. Lewis Stadler, at the University of Missouri, started work on mutations at about the same time as Muller but he chose barley as his subject and, barley being an annual plant, his results were not available until after Muller's. He is remembered as merely confirming Muller's findings [165]. Muller was awarded the Nobel Prize for Physiology and Medicine in 1946 for his discovery and subsequent exploitation of the production of

mutations by x-rays. His life after the discovery was ever turbulent: his left-wing views led him to leave Texas for Berlin in the early 1930s and then, as Germany fell into the grip of the Nazis, to Russia. Here a productive few years were brought to an end by the rise of T D Lysenko who brought Lamarkian views to dominance in Soviet genetics. He eventually managed to return to the USA during the war where he finally obtained a post at Indiana University. He remained there until he died in 1967.

A key question posed by Muller's work was the relation between the dose and the number of mutations that resulted. Hanson and Heys, by the end of the 1920s, showed that there was a proportionality between dose and mutations in *Drosophila* and that this was true for betas, gammas and x-rays [166]. Oliver, in 1930, demonstrated this over a 16-fold range so that, in his Nobel lecture in 1946 [162] Muller was able to summarise the work on radiation-induced mutations by saying that the number produced was proportional to dose down to as low as 400 r and rates as low as 0.01 r per minute for X and gamma rays.

He believed that there was no threshold dose and that the individual mutations resulted from individual "hits" that affected genes in their vicinity. The nature of these hits was undecided: they might be individual ionisations or clusters of ionisations either at the end of electron tracks or at their side branches. Whatever the hits were, the mutations resulted from "disturbances on a microscopically localized scale".

The experimental work of Muller and others on *Drosophila* was the foundation of genetics in general and radiation genetics in particular and the fruit fly continues even today to illuminate the basic physics and biology of inheritance and radiation effects. However, by the time Muller received his Nobel Prize, there was intense interest in the degree of genetic risk posed to humans by radiation and the investigation of this required something closer to us biologically than a fly. It was thus the mouse that was to provide most of the data for risk estimation in the second half of the century and the key players were the Russells at Oak Ridge, with what became known as their mega-mouse experiment, and T C Carter in England.

William Lawson Russell (known as Bill) was an English geneticist who went to the USA in the early 1930s to work on *Drosophila* and guinea pig genetics. In the late 1940s he moved to the Biology Division at Oak Ridge with his second wife and co-worker Liane Braunch and a plan to research mouse genetics. With the growing concern about fallout from weapons testing, he was encouraged to investigate the effects of radiation by the division head Hollaender – supported by H J Muller – and to think big. An entire floor of an old factory was turned over to a mouse house and the Russells started a programme on an unprecedented scale. The first results

came in 1951 [167] when just over 50 mutations were seen in the progeny of 48,000 irradiated males against two in a slightly smaller control group. The mouse house was promptly tripled in size and, with the Russells in a leading role, continued to provide the majority of the data for the assessment of genetic risk from radiation into the 1990s. It has been calculated that around seven million mice were used in the programme. This was, as Alvin Weinberg the Director of Oak Ridge said, Big Biology with “big institutes, big experiments, big money and, one hopes, big ideas.” Bill Russell died in 2003.

The key to Russell's success was not just the scale of the experiments; he also developed a simple method for detecting recessive mutations in first generation offspring of irradiated mice known as specific locus assay. This involved mating the irradiated mice with a special strain of mice homozygous for seven autosomal recessive visible mutations. Mutations of the marker loci caused by radiation will result in visible effects in the progeny.

The seven loci chosen to be followed in the specific locus test were defined by recessive mutations with visible homozygous phenotypes that were easily distinguished in isolation from each other, and had no effect on viability or fertility. The seven loci are agouti (a is the recessive non-agouti allele), brown (b), albino (c), dilute (d), short-ear (se), pink-eyed dilution (p), and piebald (s). A special 'marker strain' was constructed that was homozygous for all seven loci. [168]

A similar large-scale programme was set up after the war at the MRC Radiobiological Research Unit Edinburgh Scotland under the direction of T (Toby) C Carter before moving to Harwell in England.

In parallel with the increasing understanding of the effects of radiation, there was improved knowledge of naturally-occurring genetic disease inherited in a Mendelian way. It started with the sex-linked diseases (those from genes associated with the X chromosome) where a report on the inheritance of colour blindness was made to the Royal Society as early as 1779. In 1820 the sex-linked nature of haemophilia inheritance was recorded and somewhat later that of Duchenne muscular dystrophy.

In 1902 Archibald Garrod [169], working at the Hospital for Sick Children, Great Ormond Street, London identified the condition alkaptonuria that led to a metabolic variation that caused the urine of sufferers to turn black. Other clinical symptoms developed in later life -

among them something akin to arthritis. The condition was more common among the offspring of first cousins. With advice from William Bateson he suggested that it was an autosomal recessive trait due to single recessive gene but it was not until six years later, after further cases were studied, that this was confirmed. Garrod believed that albinism, cystinuria and porphyrinuria had similar origins and subsequently it was found that cystic fibrosis and sickle cell anaemia were further examples. In the meantime the first autosomal dominant condition in man (brachydactylyl - in which sufferers had short stubby fingers) was identified by a Yale PhD student William C Farabee in 1903 [170]. As the century progressed many more diseases were added to the list of conditions inherited in a Mendelian fashion.

The concern about the effects of bomb fallout in the early 1950s created an urgent need to assess the actual genetic risks associated with radiation. Since there were no data from humans, it was necessary to use the information from animal experiments and of spontaneous occurrence of genetic diseases in the human population. The single most important parameter for the next 50 years, one which linked these two sources, was the doubling dose (DD), the radiation dose that caused the mutation rate to double from the natural background rate.

The evolution of the ideas and the data in the second half of the 20th century is best traced through the reports of the various national and international bodies who examined the question of the genetic risk of radiation. This does not always acknowledge the origins of some of the contributions (although these can be traced in the individual reports) but illustrates how the informed and authoritative mainstream of thinking developed. Of course, it means also that we may not give enough weight to more controversial ideas that have come and, usually, gone.

The Medical Research Council (MRC) Committee on the Hazards to Man of Nuclear and Allied Radiations reported in June 1956 [45] and based its consideration of genetic effects on the DD method, starting its analysis by considering the effects that a doubling of mutation rates in one generation would have on the burden of genetic disease. Three example diseases were presented in detail based on the work of L S Penrose (described in appendices) and the results for these are summarised in Table 2.

Disease type	Sample disease	Effect of doubling of mutation rate
Dominant	Achondroplasia	80% increase in generation 1 then falling back over next few generations
Sex-linked	Haemophilia	29% increase in generations 1 and 2 then falling back over next few generations
Recessive	Phenylketonuria	1% increase in generation 1 then slow increase and decline over many generations

*Table : MRC 1956 Estimates of effects of doubling mutation rate*

The effects on a number of other mental conditions were also looked at. In its discursive style the report then gave an impression of the social impact of such rises.

It was only after this that the Committee turned to radiation and an estimate of the DD. Here they initially noted that the DD had to lie above 3 r, the cumulative dose to the gonads from natural radiation before reproduction generally ended. The human data were, of course virtually non-existent. However, it was known that radiation at the background level caused about 0.01% of the spontaneous mutations in *Drosophila*. Allowing for the longer timescales with humans and the suspected differences in spontaneous rates between man and the fly they estimated that about 2% of spontaneous mutations in man were due to radiation. There was though another correction to apply: mouse genes were known to be ten times more sensitive than fly genes. This pushed the fraction up to 20% giving a lower limit for the DD of 15 r. The upper limit, 150 r, was based on the 2% fraction.

The doubling dose information that existed for other organisms was reviewed and it was concluded that much of this pointed to values between 25 and 60 r. Combining both these sets of information the Committee put forward a best estimate for the DD of between 30 and 80 r.

At about the same time the Genetics Committee of BEAR [46] set up by Bronk reported. Its members included Hollaender, Crow, C C Little, Muller, Neel, Russell and Sturtevant and the report was prepared as the first information on the effects of radiation on mammals – notably mice in Russell's experiments - began to appear.

The main conclusions were:

- Mutant genes are usually recessive
- A small but not negligible part of the harm appears in the first generation

- There is a proportionality between dose and increased mutation rate and the effect is cumulative

Three aspects of genetic hazard concerned them:

- Risk to offspring and descendants of people receiving larger doses
- Effect of average doses on population as a whole
- Because of the increased death rate that might arise from prolonged exposure “the population, considered as a whole, would decline and eventually perish”(page 17)

Two methods for assessment were adopted: the doubling dose one and a more direct alternative. The “responsible” suggestions to the Committee for the size of the doubling dose ranged between 5 and 150 r and they settled for a statement that it almost certainly lay between these values but that it “may very well be” between 30 and 80 r . So the conclusions on DD were, reassuringly or disconcertingly, similar to the British MRC values (in fact there seems to have been collaboration). The spontaneous rate of genetic disease was estimated from the observed 4-5% of birth defects seen in the USA and it was taken that about half of these had a simple genetic origin and appeared prior to sexual maturity. There then seemed to be an assumption that almost all of these were due to background radiation because it was deduced that a DD to all the Americans then alive would lead to an increase of between two million and four million in the eventual number of defective children in subsequent generations. If the US population were subject to 10r each then there would be 50,000 defective live births in the first generation and half a million in all generations.

While this may seem comprehensible enough to us, it apparently did not satisfy all the geneticists on the Committee as meaningful. In an alternative approach six members of the Committee set out to calculate the total number of “mutants” that would result from the 10 r to the gonads. They agreed remarkably on a central value of 5 million and this was consistent with the DD calculations but they were honest enough to suggest that they could all be a factor of ten in error either way: the number could be between half a million and fifty million. This was, as the Committee put it, “disappointingly vague”. Nonetheless, the numbers were big enough to suggest that genetic effects should be the major concern- a theme that was to dominate radiological protection thinking for decades to come.

By the 1960 MRC Report [171] there was more information from Japan and much more from animals. While there were a number of

discoveries – for example of the effect of dose rate on mutation rate – overall it was clear that the previous conclusions were not far out. The rather surprising discovery in 1956 [172], after the first report, that man had 46 rather than 48 chromosomes had no effect.

The 1962 UNSCEAR report [68] reviewed spontaneous rates and concluded that one rad/generation would cause an increase of between 1/100 and 1/10th the spontaneous frequency, so keeping broadly the same DD estimate. The theme continued and in its 1966 report [173] UNSCEAR concluded that between 2 and 3% of live-born children are affected by severe disabilities arising from dominant mutations. Other conditions may also be genetic but their frequency could not be estimated.

The rate of mutations induced by radiation was estimated from experiments with mouse germ cells because the human data were “meagre” and it was estimated that there would be two mutations per 1000 male gametes per rad when the radiation was delivered as a single acute dose. While the resulting disabilities would be harmful, like the spontaneous ones, it was not possible to give an estimate of their frequency. The authors limited themselves to a statement that a dose of one rad per generation would add something like 1/70th of the total number of mutations arising spontaneously in a generation – within the previously estimated range. While the 1/70th might also apply to hereditary diseases in man it had to be remembered that there were many complexly inherited conditions also.

UNSCEAR reported again in 1972 [174] and in the same year another authoritative source of genetic risk assessment appeared: the first BEIR report from the US National Academy of Sciences [175]. In both reports the Doubling Dose method of assessing mutation risks was used: in UNSCEAR it supplemented the direct method but in the BEIR report it was adopted as the principal assessment method for gene mutations. UNSCEAR took the DD to be 100 rad for male germ cells and made no estimates for females while BEIR took a range of 20-200 rad.

UNSCEAR gave two estimates of recessive point mutation induction based on radiation-induced mutations rate of mice spermatogonia: the difference in the values obtained (a factor of 40) could be explained away on technical grounds. The increase in disease with a mutational cause that might be brought about by radiation was estimated with a DD methodology using the spontaneous incidence data from the earlier reports. BEIR used a similar indirect approach to mutations using their range of DDs.

UNSCEAR gave estimates for males but, because of the lack of data, were unable to give risk estimates for females for recessive lethals, dominant visibles and skeletal mutations. BEIR gave results for both using, where necessary, the conservative assumption that female and male risks were the

same. UNSCEAR restricted themselves to the first generation after exposure while BEIR, with some further assumptions, made estimates for both the first generation and equilibrium.

By 1976 UNSCEAR was ready to review the mouse data and its methods again [176]. The direct method was used to estimate the number of recessive and dominant mutations that might be produced by irradiation. Recessive mutation rates were estimated as  $60 \times 10^{-6}$  per gamete per rad for irradiation of sperm and much lower for female germ cell irradiation. The reduction factor of three, used in their previous report to allow for the low dose rate applicable to human exposure compared with that in the mouse experiments, was confirmed and used again.

The risk of dominant mutations that might lead to serious handicap was estimated from the rate of induction of skeletal mutations in the offspring of irradiated mice. The risk of these skeletal mutations (about  $4 \times 10^{-6}$  per gamete per rad) was adjusted to allow for about 10% of dominant mutations in man being associated with skeletal abnormalities and about 50% of these dominant mutations causing serious handicap. Overall this led to a dominant mutation risk associated with serious consequences of about  $20 \times 10^{-6}$  per gamete per rad for male irradiation. No estimates were made for female irradiation but the risk was considered low.

The doubling dose method was also used for estimating the increased incidence of Mendelian diseases taking spontaneous rates as 0.1% for recessive diseases and 1% for dominant and X-linked diseases. With the doubling dose of 100 rad derived from mouse experiments (and now indicated as a minimum value by data on the mortality of children born to bomb survivors) they estimated the impact of 1 rad per generation of low-LET radiation. There would be, at equilibrium, about 100 additional cases per million births of dominant and X-linked disease. In the first generation, per million live-born, there would be about 20 additional cases: not much different from the direct estimates. However, they did comment (para 637) that one of the main assumptions of the doubling dose method, that of the proportionality between spontaneous and induced rate, "still remains to be proved".

In the report published in 1983 [75] UNSCEAR essentially confirmed its conclusions in 1977. The knowledge the occurrence of spontaneous mutation diseases was more firmly established and the animal data had expanded considerably both in terms of species and irradiation conditions. The lower sensitivity of female germ cells was confirmed and there was support for the basic proportionality assumption of the doubling dose method from fruit flies and bacteria. The estimates from the doubling dose method of the increased equilibrium incidence of dominant and X-linked

disease remained unchanged from 1977 but the number expected in the first generation reduced slightly. In summary – and in the new Gray units – the incidence of mutation-related diseases in a population of one million exposed to 1 Gray per generation would increase to 1500 in the first generation and 10,000 at equilibrium. Such diseases would then account, as we will see, for some two-thirds of the total genetic impact.

The 1986 UNSCEAR report [177] saw some further adjustments to the assessed mutation risks. Most significant perhaps was a somewhat lower dominant mutation risk for males as indicated by further mouse data. It was also possible to make an estimate of the risk from induced autosomal recessive mutations (which in previous reports had been assumed negligible). For our one million population and a single dose of 1 mGy, there would be no extra cases in the first generation and possibly one extra one in the following ten generations.

The 1993 UNSCEAR report [178] pointed to three conditions that confused inheritance. Mosaicism, when both normal and mutated germ (and somatic) cells can be present in the same individual, genomic imprinting, where the expression of genes sometimes depended on whether they had been inherited from the mother or father and uniparental disomy, when all the chromosomes came from one parent.

The 2001 UNSCEAR report [179] was the first to take advantage of the advances of the late 20th century in molecular biology which, in many cases, complicated the treatment of Mendelian diseases. The one-to-one relationship between genetic make-up (the genotype) and the physical make-up (the phenotype) had broken down: there were mutations that could lead to several diseases; some similar diseases came from quite different gene mutations and genes that were dominant in some people were recessive in others. Allelic expansion (where a gene expands as it is transmitted between generations) is also an example of effects that greatly increased complexity. It was also recognised that the division of genetic damage into gene mutation and chromosomal damage was a fairly arbitrary one: point mutations were at the molecular end of a continuum that extended up to the damage visible through a microscope. Although the distinction was formally maintained, in fact chromosomal disease was subsumed under Mendelian diseases and congenital disease.

The key risk estimates were made using the doubling dose method based on the spontaneous disease rates in man (rather than the mouse) but taking the doubling dose itself from mouse experimentation. The concept of the Mutation Component (MC), initially developed by Crow and Denniston [180] in the 1980s, was developed as a mathematical model in the 1990s with the MC defined as the fractional increase in disease per

fractional increase in mutation rate.

With this additional factor the risk equation became

$$\text{Risk per unit dose} = P \times (1/DD) \times MC$$

Where P is the background incidence of the disease.

For the dominant Mendelian diseases MC is related to the selection coefficient,  $s$ , the probability that an individual with a genetic disease will go on to reproduce. For these diseases the Mutation Component value can be estimated for a variety of situations using standard population genetics models. For example, for a one-time increase in mutation rate (a so-called burst) the Mutation Component for the subsequent  $g$ th generation is:

$$MC(g) = s(1-s)^{g-1}$$

The value chosen for  $s$  by UNSCEAR [181] was of the order of 0.3 and this defined the MC for first generation risks. This value was used in making the risk estimates for autosomal dominant and X-linked diseases in their 2001 Report [181] and the following BEIR. The value for autosomal recessive diseases is expected to be near zero.

The 1990s saw the beginnings of the resolution of one of the persistent problems of genetic risk: the squaring of mouse data with the fact that, as Sankaranarayanan put it in a 2002 paper [182], “no radiation-induced germ-cell mutation, let alone an induced genetic disease has been found in humans!”. The results from the children of bomb survivors [183], at the beginning of the decade, began to suggest that the doubling dose of about 1 Gy derived from the extensive mouse experiments, might be several times too low. A better understanding of the nature of mutations made it clear that the source of the discrepancy might lie in essential differences between spontaneous mutations and those cause by radiation. They were different in nature: those caused by radiation were largely deletions of single or multiple genes while spontaneous ones were a mixture of types. They were differently caused: radiation damage arose at random while the spontaneous mutations were often closely related to DNA sequence organisation. And they had different effects: the radiation-induced changes generally caused a loss of gene function while the spontaneous ones sometimes resulted in a gain of function. These new complexities made the simple concept of the doubling dose a good deal shakier than before. Specifically, they suggested that the mutation caused by radiation, that might lead to genetic disease, might also frequently lead to the non-viability of the genetic material. If this

were so, the genetic changes would never be expressed in live births and they would be lost. In the jargon, they would not be “recoverable”.

This interpretation seemed to offer an explanation of the differences between the mouse experiments and the experience with humans. The doubling dose had been deduced from the mouse experiments and most recently from work on recessive mutations in just seven genes. But these were subject to a bias since these genes were chosen for study because they were not essential to survival of the animal and were in a non-essential region of the genome: they were chosen just because they were “recoverable”.

It was clear that it was necessary to estimate just what the impact of this was and this was done, in the late 1990s, by Sankaranarayanan and Chakraborty [184]. They estimated, in a detailed study based on experience with mice, the expected impact of a radiation-induced deletion on 63 autosomal and X-linked human genes that between 15 and 30% might be recoverable and be capable of causing genetic disease. They termed this the Potential Recoverability Correction Factor, PRCF and thus extended the basic risk equation to:

$$\text{Risk per unit dose} = P \times (1/DD) \times MC \times \text{PRCF}$$

The approach was adopted by both UNSCEAR [181] and BEIR.

Because of the better understanding of the nature of genetic disease, its spontaneous occurrence was recognised as being higher than previously thought: overall, estimates nearly doubled to 240 cases per 10000 live births from the figures used previously. However, the more detailed mathematical treatment led to estimates for autosomal dominant and X-linked diseases that were not very different from those of 1993. With the 1 Gy doubling dose, the estimates for parental exposure of 1 Gy to low-dose low-LET radiation are 750-1500/million in the first generation and 1300-2500/million progeny in the second. The risk of recessive diseases was put at zero. The Committee’s estimates were limited to the first two generations to recognise the uncertainties in the demographics and the various parameters used in the risk assessment over longer period of time.

The evolution in the last half-century of the estimates of the risks of Mendelian diseases due to radiation exposure from the different sources are summarised in Table 3. While a few assumptions have been made here in deducing single figures to represent the outcome of complicated arguments

	Risk per million liveborn/Gy per generation		
	Autosomal dominant and X-linked		Recessive
	1 <sup>st</sup> generation	Equilibrium	1 <sup>st</sup> generation
BEAR 1956			
UNSCEAR 1962		1000-10000(c)	
UNSCEAR 1966		1300(b)	
UNSCEAR 1977	2000 2000(a)		
UNSCEAR 1983	1500 1000(a)	10 000	
UNSCEAR 1986	500-1000		0-
BEIR-V 1990	500-2000 severe 100-1500 mild	2500 severe 7500 mild	<100
UNSCEAR 1993	1500		~5
UNSCEAR 2001	750-1500(d)	No estimate made	0

a) based on direct method

b) 0.1%(para 4) x 1/70 per rad (para 25)

c) based on 0.1% incidence and 1/100-1/10 natural incidence per rad

d) includes some diseases previously classified as chromosomal

*Table: Mendelian disease risks from radiation*

and there have been changes in classification over the last fifty years, the consistency is striking. Perhaps it should not be surprising though; the doubling dose has remained about 100 r (in old units) and the estimates of background rates have not really not changed significantly.

The Mendelian effects discussed to this point have arisen from mutations and these are associated with changes in just a single gene. It is the fact that some such changes lead to viable offspring who can pass on the mutation that allows it to persist. Such point mutations are, however, just one end of the spectrum of damage that radiation can cause in chromosomes.

Changes in entire sets of chromosomes in plants had long been known and these had confused early workers (as noted earlier on the origins of the word “mutation”). However, more relevant from our point of view were the results that emerged from *Drosophila* in the 1920s where, to explain the

observed gene linkages, workers were forced into assumptions about the behaviour of chromosomes. Three broad types of gross damage were postulated to occur spontaneously: deficiency (a length of chromosome was lost), inversion (a piece was removed, turned around and put back) and translocation (a piece was transferred to another chromosome).

To some these invisible changes were just speculation and an easy way to explain anomalous results. However, in 1933, T S Painter discovered the gigantic chromosomes in the salivary glands of *Drosophila* larvae that were large enough to study under the microscope and make detailed maps of the banding. Soon it was possible to tie the locations of genes predicted by purely genetic means to the bands on the chromosomes and, in a striking confirmation of the insight of the earlier workers, the various presumed types of changes to chromosomes could now actually be seen. They were real. It was later found that the giant chromosomes had been known to cytologists in 1881; it had taken 50 years for geneticists to find them and exploit them in these critical observations. [161]

The mechanism by which radiation created these changes was not really understood until the 1930s. Early work assumed that, after a chromosome was broken, the “centric part” (that with the centromere) could be passed down through cell divisions as a fragment. These and other fragments would join up in different ways to cause the postulated damage. It was H J Muller in 1932 who developed the picture that fitted the majority of cases: the *Drosophila* chromosome was actually broken in two places and these ends were then rejoined in the different ways. The original free ends of chromosomes were always free; they were specific structures, telomeres, defined by the genes there. Muller’s idea was based on a wide range of experimental data and by 1938 he was convinced that this was the general model of structural change. [164]. By 1938 he was also sure that the breaks that were the source of the damage were independent – based on the dependence of the frequency of their occurrence on the 1.5th power of the radiation dose that caused them [164]. Similar results came from Sax with plants [185] and Carlson [186] with grasshoppers.

On the surface a squared relation with the dose was to be expected but Muller explained his results by the increased cell killing that occurred at higher doses and the various susceptibilities among the exposed cells. He also noted that where the radiation was densely-ionising fast neutrons the relation was proportional, suggesting that two breaks were produced close together by the same track and joined. [187,188].

By 1956 the MRC Report [45] was able to summarise the effects of

chromosomal damage (page 28):

1. Structural changes are caused most readily by large acute doses of radiation such as x-rays and from atomic bombs; extended exposure to low intensity x-rays only rarely causes them although neutrons and alpha particles are more effective
2. Chromosome breakage usually results in cell or embryo death
3. But, if fragments reunite in new patterns, these may be capable of passing through cell division
4. The resulting structurally changed chromosomes may be transmitted to apparently normal offspring and one type of change may lead to repeated abortions or malformations in descendants
5. Major chromosomal changes may bring sterilisation or abortion but do not, as a rule, cause abnormalities

It has become apparent that spontaneous chromosome damage is not uncommon. A high proportion of abortions and about 0.5% of live births show some kind of chromosome anomaly with the most common being trisomies followed by unbalanced translocations. Trisomies are extra chromosomes which result from the failure of chromosomes to separate properly (non-disjunction) in meiosis and occur in several chromosomes. Down's syndrome results from the presence of an extra chromosome 21 (Trisomy 21), but Trisomy 18, Trisomy 13, Turner syndrome (a missing or incomplete X-chromosome in women), and Klinefelter syndrome (an extra X chromosome in men) are also seen in live-born infants.

However, the conclusion of the 1956 MRC committee [45] has remained broadly valid: "chromosomal structural changes are likely to be of comparatively little importance among the radiation hazards to man" (para 125)

In spite of the time that has been spent in this chapter on Mendelian diseases, most traits or diseases with a genetic component are not inherited in such a simple way; they are associated with more than one gene (they are polygenic). Their expression is also not determined solely by genetics but by environmental factors and they tend to run in families. Normal traits that behave like this include stature, intelligence and skin colour. Abnormal conditions such as common congenital disorders, diabetes mellitus, rheumatoid arthritis as well as some psychiatric conditions like schizophrenia are inherited in this way. While in multifactorial inheritance (as it became known) the individual genes are inherited in a Mendelian fashion, their combination and the folding in of environmental factors is far

more complex and requires a different approach

In normal traits the variation caused by multifactorial inheritance shows up as a normal (or Gaussian) distribution of an attribute such as stature but for abnormal conditions it is the likelihood of occurrence of the condition which is important. To predict this Falconer [189] in 1965, using earlier concepts of Wright [190,191], defined a liability: an unobservable continuous parameter determined by an additive combination of genetic and environmental factors. This liability is then expected (on the basis of the central limit theorem) to be normally distributed and, in the so-called threshold model, should its value be greater than a certain threshold value, then the individual will develop the abnormality or disease.

The application of these ideas to risk estimation has been fraught ever since: it has been difficult to establish which diseases have a genetic component and just how big that component is and, beyond this, there have been varying estimates of spontaneous rates. Since the spontaneous rates have generally formed the baseline for the assessment of any enhancement of risks from radiation exposure, these assessments have usually been restricted to congenital abnormalities where there is some agreement on the background occurrence. If there is an overall pattern it is of early confidence followed by pessimism and then a cautious and measured optimism.

UNSCEAR 1966 [173] and the BEIR 1972 report [175] both made estimates of spontaneous occurrence of multifactorial diseases and by 1977 UNSCEAR [176] was confident enough to make some estimates of radiation risk based on a doubling dose of 1 Gy and a mutation component of 5%. These were reiterated a few years later in 1982 [75] but by the time of its 1993 report [71] there was a rethink:

...the uncertainties did not justify continuing this procedure, given the higher estimated incidence of these conditions of varying seriousness that can arise throughout a lifetime [71] para296

It seemed to say that while the methodology was satisfactory while the results were rather insignificant, now the prevalence of MF disorders was understood to be high it could not be trusted. Estimates of radiation risks for these disorders were not made by UNSCEAR between 1982 and 2001 but evidence showing that multifactorial diseases were rather common continued to grow

The UNSCEAR 1986 [177] report divided multifactorial disorders into congenital abnormalities and others. The background rate for congenital abnormalities used in the 1982 report [75] (and based on British Columbia

data) was 430/10,000 live births. By 1986 it was possible to re-evaluate this using data from Hungary and the United States that had not been fully and systematically taken into account in the earlier report. The use of data from a variety of sources proved, as might be expected, difficult and the conclusions were rather complex. However, broadly, the prevalence was between about 430 and 850/10,000 live births depending upon exactly which categories of abnormalities were included. The Committee decided upon 600/10,000, derived from the Hungarian data but representing a realistic average of the available information.

From the British Columbia data, a rate of 470/10,000 had been derived by the workers Trimble and Doughty but this was really only for disorders that showed up before the age of 21 and was therefore clearly an underestimate. Hungarian work, just becoming available, suggested a much higher value: 6000/10,000. The proportion of people affected was actually less than 60% since many suffered from more than one defect. The Committee was unable to make any comment on the effects of radiation.

The 1988 report [192] could add little to the previous one and UNSCEAR 1993 [71] did not adopt an optimistic tone in discussing multifactorial disorders. Rather it detailed the many uncertainties and complexities that stood in the way of an adequate treatment. The spontaneous rates were essentially unchanged, the availability of mathematical models for multifactorial disorders was noted but the conclusion on radiation was not encouraging:

...in view of the complex genetic basis of most malformations and of multifactorial diseases, it is impossible to estimate increases caused by radiation... [71] para 296

The 1990 BEIR V report [193] arrived at a lower value for spontaneous congenital abnormalities than the figure adopted by UNSCEAR in 1986: 20-30 per 1000 live births rather than 60. However, there was an enormous increase in the number of other multifactorial diseases (or as BEIR preferred “disorders of complex etiology”) from 600 per thousand to 1200 per thousand. These were broken down into heart disease (600), cancer (300) and “selected others” (300). This means that there are more disorders than births.

The conclusions at various time on spontaneous rates are shown in Table 4.

In contrast to UNSCEAR, BEIR did make some estimates of the impact of radiation – but only on congenital disorders. They concluded that there would be one additional case seen in the first generation per

thousand

Estimated spontaneous burden of multifactorial disorders (per 1000 live births)		
Source	Congenital Abnormalities	Other multifactorial
Stevenson 1959	10	10
UNSCEAR 1966	25	15
BEIR 1972	15	25
Trimble & Doughty	43	47
UNSCEAR 1977	90	
Carter 1977	24	-
BEIR 1980	90	
UNSCEAR 1982	43	-
Czeizel & Sankaranarayanan 1984	60	-
UNSCEAR 1986	60	600
UNSCEAR 1988*	60	600
BEIR V 1990	20-30	1200
UNSCEAR 1993*	60	650
UNSCEAR 2001*	60	650
BEIR VI (draft)*	60	650
From BEIR V Table 2-5 except * from original documents. All rounded to nearest whole number. UNSCEAR 1977 and BEIR1980 are combined values		

*Table: The background of multifactorial disorders*

live births per Sv per generation and between 1 and 10 per thousand in each generation at equilibrium. A doubling dose of 1 Sv and a mutation component of 5-35% was used.

The work through the 1990s on Mutation Component and, latterly, PRCF that contributed to the understanding of the risks of pure genetic diseases finally made it possible to construct some estimates of the enhanced risks from multifactorial diseases caused by radiation. The ICRP Task Group set up to consider multifactorial diseases was able to report in 1999 [194] with a structured approach to making the estimates.

To estimate MC they used a hybrid model that became called the Finite Locus Threshold Model (FLTM) which combined the threshold model for liability with a mechanistic population genetic model. The threshold model described above assumed that the number of genes involved was very large so that the liability could be expected to have a normal distribution. The Task Group took a rather different approach: they assumed that there were

just a few genes (actually five) responsible for the genetic component. The environmental contribution was assumed to be normally distributed.

The model was then subjected to what was essentially a sensitivity analysis to see how, for a plausible range of parameters (such as  $s$  and the threshold level), the Mutation Component varied with heritability. The results were complex but some simplifying principles emerged. The most important was the discovery that, in the early generations, MC was very much less than 0.02 for heritability between about 30% and 80%. Multifactorial diseases were thus predicted to be “far less responsive to induced mutations than Mendelian diseases” [181]. The factor 0.02 was adopted for risk estimation for chronic multifactorial diseases by UNSCEAR [181] and BEIR [108]. Conclusions are summarised in Table 5.

Source	Effect of 1 Sv (low-LET) per generation/ $10^6$ live births/generation	
	First generation	Beyond
UNSCEAR 1977	used DD 1 Gy+MC 5%	
UNSCEAR 1982	used DD 1 Gy+MC 5%	
BEIR V 1990	1000(n/e)	1000-10000(n/e) at equilibrium
UNSCEAR 1993	n/e(n/e)	n/e(n/e)
UNSCEAR 2001	~2000(~250-1200)	2400-3000(~250-1200) in second generation
n/e: not estimated. Main figure for congenital disorders, “other multifactorial” in brackets		

*Table : Radiation risk estimates for multifactorial diseases*

Chromosomes were known from rather early in the century to be made of proteins and DNA, a long stringy molecule that was thought to be some kind of constructional material. Until 1944 almost all scientist thought that the genes were somehow located on the proteins but, in that year and after decades of incremental research work, a team at the Rockefeller Institute for Medical Research in New York led by Oswald Avery showed that they were in fact carried by the DNA. It was not an instant success as a theory, partly because the work was actually on the transformation of bacteria and partly because DNA seemed too simple a molecule for this awesome task. For another ten years the matter was not settled; the crucial illumination of the molecular basis of the genes had to wait for the development of the science

of x-ray crystallography and the imaginative genius of James Watson and Francis Crick. Their double helix model of DNA, published in 1953, answered two key questions: how is the genetic code carried and how is it passed on? One of the great insights of 20th century science, it has explained in molecular terms all the observations of genetics in the previous century and has been a fertile agent of new ideas ever since.

It has allowed us to track down the molecular changes that cause (or predispose us towards) many diseases. But it has also shown us that nature is even more complex than we thought and much more messy. The DNA molecule is largely composed of apparently meaningless repetitive sequences interrupted by seemingly randomly-located stretches that carry instructions. It was, of course, created and modified by natural selection and, as Professor Steve Jones puts it in his book *The Language of the Genes*: “Natural selection has superb tactics, but no strategy” [195]. It is a process that has acted “like a handyman rather than a craftsman” leaving “products that are badly – not to say extravagantly –planned and roughly made”.

Nonetheless, we now know a great deal about the molecular nature of our genes, how they are passed between generations and how they are used to construct the proteins that make up our bodies. We have witnessed one of the great revolutions of science but, by the end of the century, the molecular nature of the genes had had surprisingly little impact on the understanding of the action of radiation in genetic terms. Our assessments would have been little different even if our knowledge had been much less.