THE EXPERIMENTAL PRODUCTION OF MUTATIONS

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CONTENTS.

I. INTRODUCTION.

The possibility of influencing, or even directing, the heritable variability of organisms is unquestionably one of the central problems of biology. And we know that biologists, and especially the evolutionists, were already interested in it at the end of the past century. But until recently this problem was unfortunately entangled in a morass of different more or less speculative evolutionary deductions. Even experimental work was influenced chiefly by the desire to prove or disprove the Lamarckian theory of the "inheritance of acquired characters"; in most cases it had already influenced the premises of the experiments in such a way that it was quite hopeless to draw any exact conclusions from the results obtained. This type of work will not be reviewed here. Only a short critique of it will be given, in order to show what kind of technical errors should be avoided in experiments on production of mutations.

Still another group of experiments will be omitted in the present review, as it has only an indirect connection with genetic problems: the histo- and cytopathological work done on treated germ cells. Since the first classical experiments of Gerassimow (1901), in which he succeeded in inducing the doubling of the set of chromosomes in *Spirogyra* by cold treatment of the cells, much work was done on the experimental influence of different agents (especially X-rays and radium) upon the nuclei of cells. But in almost all cases, except the recent work done by geneticists using suitable material, no genetic tests or evaluation of the induced chromosome variations were made. The most important results of this kind of work have already been summarised in another review (P. Hertwig, 1927).

Thus, within the limits of the present review there will only fall genetic experiments *sensu stricto* on the experimental production of heritable variations, performed on suitable objects and using exact technique. In the discussion of the principal questions, work on *Drosophila* will occupy a dominant position; not because the present author, being himself a *Drosophila* geneticist, is better acquainted with this material, but because of the actual conditions in experimental genetics, and of the fact that *Drosophila* has many advantages for exact genetic experiments.

II. A CRITIQUE OF OLDER EXPERIMENTS.

As has already been mentioned, we will not review in detail the older experiments dealing with the "inheritance of acquired characters," since they have already been reviewed several times (Semon, 1912; Kammerer, 1925; Wladimirsky, 1927). These experiments, if analysed critically, all show that no results were obtained at all. This is true even for the most recent experiments of this kind (Guyer and Smith, 1920; Dürken, 1923; Wladimirsky, 1929), performed at a time when the knowledge of modern genetics was already widely spread.

The reasons why all these experiments are of no scientific value are the following: (1) unsuitable material, (2) inexact technique, (3) small numbers of tested individuals and cultures, and (4) absence of exact genetic knowledge of the "normal" and "induced" variations in the tested objects. It is astonishing how obtusely all these experiments were planned and performed. The most inconvenient objects (*e.g.* salamanders or genetically quite unanalysed butterflies) and complicated or absolutely unanalysed reactions (*e.g.* formation of lens antibodies or formation of conditioned reflexes) were often used in this kind of experiment. The technique of treatment, as well as the breeding methods of the treated and control material, were in almost all cases insufficient to give sound results. Under the influence of the idea of "somatic induction" most of the authors did not concern themselves at all about the question of the penetration of the applied agent into the germ cells of the treated individuals. The breeding (genetic) methods and the numbers of tested individuals were such that, even in the cases with positive results of the treatment, the effect on mutability could not possibly be detected. And, last but not least, the genetic knowledge about the organisms taken as experimental objects was very poor: the cultures were not sufficiently inbred to secure pure and homogeneous material, the normal (spontaneous) rate and the kind of heritable variations

appearing in the material were quite unknown, and the characters said to be induced were not analysed genetically in an adequate way.

The chief theoretical error of almost all of these experiments was the attempt to solve directly certain evolutionary problems, without consideration of the many purely genetic (and sometimes even physical) questions and details, which must be analysed and solved in any experiment dealing with the artificial induction of genotypic variations, before any general conclusions can be drawn.

The older experiments have thus only a negative significance: they are a kind of warning, showing us the mistakes which we have to avoid, and the precautions that we have to take in planning and performing experiments on the induction of heritable variations.

III. CRITERIA OF THE TECHNIQUE FOR EXPERIMENTS ON THE PRODUCTION OF MUTATIONS.

In order to be able to reach conclusive results the following requirements must be fulfilled in performing any experiment on the production of heritable variations.

The *first requirement* is the genetic purity of the material used in the experiments. The stock from which the control and treated material is to be taken must be genetically analysed and closely inbred for several generations at least. Wild populations, as well as laboratory stocks kept for long periods in unanalysed mass cultures, may already contain various mutations in the heterozygous condition (H. A. and N. W. Timoféëff-Ressovsky, 1927; Tschetverikov, 1928). After being inbred in the course of the experiments they will show recessive "mutations," not freshly arisen but due to the segregation of some of the mutant genes already present in some concentration in the original stock.

The *second* and *third requirements* are: sufficiently large numbers of individuals and cultures in both the controls and the treated material, and genetic methods (types of crossings) suitable for the detection of newly arisen mutations. These two requirements are intimately connected with one another. It is self-evident that the numbers must be large enough to give sound results; but they must also correspond to the number of treated gametes. If five mice (*e.g.* males) are rayed and crossed to five untreated females, and say thirty F_1 individuals are raised, it will mean that only thirty treated gametes can be analysed in further generations. It must also be remembered that autosomal recessives will in most cases not show themselves before F_3. Thus, sufficiently large numbers of F_1 individuals (corresponding to the number of treated gametes) must be bred and these F_1 individuals must be adequately analysed (at least until F_3) in order to discover whether they contain newly arisen mutations. The criteria of experiments for the induction of mutations fulfilling these requirements have been described in a special paper by P. Hertwig (1932 *b*).

The *fourth requirement* is the exact analysis of the variations arising, which can be of different types: (1) non-heritable modifications, (2) plasmatic enduring modifications (*Dauer-modifikationen*), (3) gene mutations, (4) chromosomal abnormalities. An exact analysis can only be performed if a genetically suitable organism has been chosen as the object of the experiments.

The *fifth requirement* is some knowledge concerning the manner in which the agent used can act on the germ cells of the treated object. Such agents as, for instance, X-rays or γ-rays always penetrate directly to the chromosomes of the treated germ cells. But in many other cases it must be demonstrated that the agent used can really reach the chromosomes of the germ cells of the object (*e.g.* in the cases of ultra-violet rays, visible light, or chemical treatments). It is certainly not impossible that some agents, although unable to penetrate directly to the germ cells, can nevertheless cause mutations indirectly by certain chemical changes induced primarily in some other parts or tissues of the treated organisms. But, in any case, this must so far as is possible be proved.

All the above requirements must be fulfilled in experiments designed to establish new viewpoints or to solve the principal questions in genetics. But the fulfilment of the first three of these requirements is absolutely necessary in any experiment dealing with the influence of any agent whatever on the heritable variation of organisms.

IV. RADIATION GENETICS.

In the problem of the experimental induction of heritable variations, radiation genetics—the production of mutations by short-wave radiations—occupies, both quantitatively and qualitatively, not only the first, but almost an exclusive place. Not only do most of the exact experiments on the induction of mutations lie at present within this field, but also the most interesting theoretical attempts to analyse the nature of the gene and of the process of mutation are connected with the use of short-wave radiations. Thus, radiation genetics will also occupy by far the most important place in the present review.

(1) *Historical attempts, and the first experiments of H. J. Muller.*

History. Soon after the discovery and elaboration of the physical properties of X-rays and radium, biologists and physicians emphasised the importance of these agents in attempts to affect the internal delicate structures of cells and tissues. Special histopathological work was done first by Bardeen (1906) and then by Regaud and Dubreuil (1908) and by O. Hertwig (1911, 1913) and his associates on animal germ cells and by Gager (1908) and Guilleminot (1908) on plants. Since then a great deal of work has been done in this direction showing that different structures in cells, including the chromosomes, can be affected by X-rays and radium. But, as was already mentioned, no strictly genetic tests were made in any of these experiments.

As early as 1920 some strictly genetic experiments with X-rays and radium were started. The most conclusive results were obtained by Nadson and Philippov, who succeeded in inducing new stable races of fungi (Nadson, 1920, 1925; Nadson and Philippov, 1925, 1926, 1928, 1931, 1932). Stein succeeded in inducing *Radiomorphose*, a cancer-like tissue abnormality, in *Antirrhinum majus* by radium treatment (Stein, 1922, 1926, 1927, 1929, 1930). This abnormality proved to be heritable

(Stein, 1932 *b*). Special tests on mice were performed by Bagg and Little and by Dobrovolskaia-Zavadskaia in order to induce mutations by X-rays (Little and Bagg, 1923; Bagg and Little, 1924; Dobrovolskaia-Zavadskaia, 1928). But the results obtained were inconclusive, although several mutations were probably induced by X-rays.

Approximately at the same time another series of experiments was started upon the influence of X-rays and radium on crossing-over and on non-disjunction of the *X*-chromosomes in *Drosophila melanogaster*. Mavor showed that X-rays influence the percentage of crossing-over in the *X*- and the II-chromosomes and raise the percentage of non-disjunction in this species (Mavor, 1921, 1922, 1923, 1924; Mavor and Svenson, 1924). He also showed that these effects are due to a direct influence exerted by the X-rays on the chromosomes of the germ cells (Mavor, 1929). Plough (1924) found that radium produces the same effects as X-rays on crossing-over and non-disjunction. Muller studied the differential effects of X-rays on crossing-over in different parts of the *X*-, II-, and III-chromosomes in *D. melanogaster* (Muller, 1925, 1926), and succeeded in producing genetically detectable chromosome breakages in the same species by X-rays (Muller and Dippel, 1926).

But all the above experiments, although showing that the germplasm can be affected by short-wave radiations, did not solve the problem of artificially inducing mutations, because they were either performed on unsuitable material and with inexact technique, or (as the *Drosophila* experiments on crossing-over) they dealt with other special problems. The question of the production of mutations by short-wave radiations was first definitely solved by Muller's X-ray experiments on *D. melanogaster* (Muller, 1927, 1928).

H. J. Muller's experiments. Muller's discovery of the pronounced effect of X-rays on the process of mutation was not a matter of chance, as has been the case in many other discoveries. His success was rather due to a very thorough and ingenious theoretical and technical preparation of the experiments. His experiments were the first which exactly fulfilled *all the requirements* enumerated above, showing at the same time that these requirements *must* be fulfilled in any exact experiment dealing with induction of mutations.

Since 1919, Muller has studied quantitatively the normal, spontaneous process of mutation in *D. melanogaster* (Muller and Altenburg, 1919; Muller, 1923, 1927 *a*, 1928 *b*). With the help of specially adapted, exact breeding methods he was able to show that it is possible to detect all sex-linked mutations arising in a certain number of gametes. He found that the rate of mutations in the *X*-chromosome of *D. melanogaster* is measurable and equals about 0·1 per cent. By far the majority of the detectable mutations were found to be recessive lethals, producing no effect in the heterozygous condition, but killing the organism if homozygous. Muller synthesised special cultures and described a method of crossing which allowed the easy and exact detection of all the sex-linked lethals arising in the sperm cells. Using exact methods of breeding and taking into consideration the whole experience and knowledge of *Drosophila* genetics accumulated since the beginning of the

genetic work with this species in Morgan's laboratory (Morgan, Bridges and Sturtevant, 1925), Muller could perform his X-ray experiments most critically.

His technique was as follows. Flies containing one or more mutant genes in their *X*-chromosome as markers were X-rayed in small gelatine capsules (50 kV., 5 mA., 1 mm. aluminium, dosage varied) and then crossed to untreated flies with a different constitution of the *X*-chromosomes. In the progeny of these crossings the treated (and marked) *X*-chromosomes could be followed and all mutations which arose in them during treatment could be detected.

Of special importance are two methods of crossing, which now are used in all

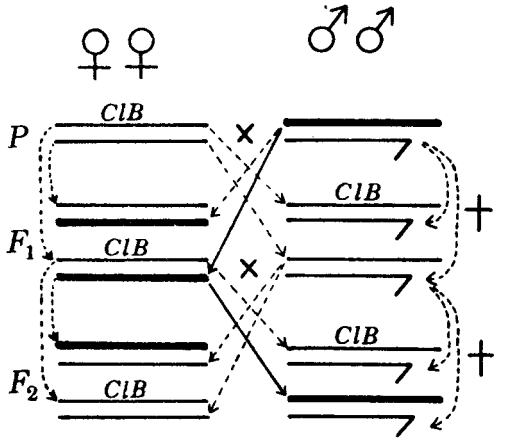

Fig. 1.

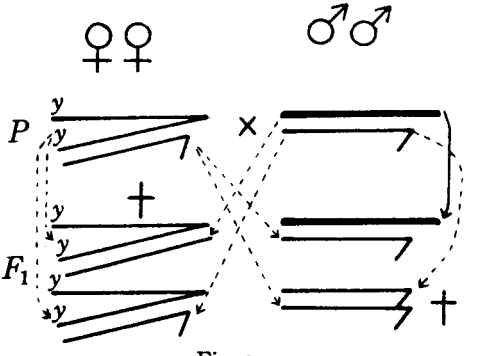

Fig. 2.

Fig. 1. Scheme of the *ClB* crossings in *D. melanogaster*; these crossings are especially suitable for the detection of induced sex-linked lethals. One of the *X*-chromosomes of the females contains an inhibitor of crossing-over (*C*), a recessive lethal killing the males (*l*) and the gene Bar as marker (*B*). *P* ♂♂ are rayed and in F_2 of *ClB* F_1 ♀♀ arising from sperms which contain a newly arisen lethal no males will appear at all. The rayed *X*-chromosomes are represented by darker lines.

Fig. 2. Scheme of the *attached-X* crossings in *D. melanogaster*; these crossings are especially suitable for the detection of induced sex-linked visible mutations. The two *X*-chromosomes of the females (containing the recessive gene yellow body colour as marker) are fused at their spindle-fibre ends; all surviving F_1 ♂♂ get their *X*-chromosome from the father. *P* ♂♂ are rayed and all visible sex-linked mutations induced in their sperm cells will show in the F_1 ♂♂. The rayed *X*-chromosomes are represented by darker lines.

exact mutation experiments with *D. melanogaster*: (1) the *ClB method*, and (2) the *attached-X method*. The scheme of the first of these is shown in Fig. 1. The P_1 ♀♀ contain in one of the *X*-chromosomes a dominant inhibitor of crossing-over (*C*), a recessive lethal (*l*) killing the males which contain this chromosome, and the dominant gene *Bar*-eye (*B*) as marker; such females give in their progeny a 2 : 1 sex ratio, since half of the males (*ClB*) die. If P_1 ♂♂ are treated and a large number of F_1 *ClB* ♀♀ are tested by further crossings, all mutations arising in the treated and tested *X*-chromosomes will show in F_2 ♂♂; if lethals arise in the treated *X*-chromosomes, the corresponding F_2 cultures will give no males at all (because one-half of the males will be killed by the *ClB* chromosome and the second half by the new lethal). The other method is shown in Fig. 2. The *attached-X* ♀♀ ($\widehat{XXY}$) have both

their X-chromosomes fused at the spindle fibre ends and possess an extra Y-chromosome; half of the eggs formed by these females contain the attached X-chromosomes ($\widehat{XX}$) and the other half the Y-chromosome. The eggs with attached X-chromosomes, if fertilised by X-containing sperm, give inviable $\widehat{XX}X$ super-females (a few of them sometimes survive, but are sterile); and if fertilised by Y-containing sperm, give *attached-X* females ($\widehat{XX}Y$). The eggs with a Y-chromosome give, when fertilised, either the inviable YY combination or regular XY males. All sons of the *attached-X* females thus get their X-chromosome from the father; if the P_1 ♂♂ are rayed all visible mutations produced by the treatment in the X-chromosome of their sperm cells will already be detectable in the F_1 males. The *attached-X* culture was first found and described by L. V. Morgan (1922) and is

Table I. *Results of the "first X-ray experiments" of Muller with* D. melanogaster (50 *kV.*, 5 *mA.*, 1 *mm. aluminium*, 16 *cm. distance;* $t_1 = 12$ *min.*). (*From Muller*, 1928 *c.*)

Series	No. of P_2–F_2 cultures		No. of sex-linked mutations			
	Started	Hatched	Lethal	Semi-lethal	Weak	Vigour
Controls	1011	947	1	0	0	0
X-rays t_1–t_4	1015	783	91	17	9	11

Table II. *Results of Muller's* ClB *experiments. Males were* X-*rayed* (*dosages* t_2 *or* t_4) *and mated with* ClB *females.* (*From Muller*, 1928 *c.*)

Series	No. of fertile P_2–F_2 cultures	No. of sex-linked mutations		
		Lethal	Semi-lethal	Visible
Controls	198	0	0	0
X-rayed, dosage t_2	676	49	4	1 +
X-rayed, dosage t_4	772	89	12	3 +

best suitable for the detection of visible sex-linked mutations induced in treated sperm cells.

Table I shows the results of Muller's first X-ray experiment (1928 *c*). The number of mutations in the X-rayed chromosomes was found to be about 150 times higher than in the controls (128 : 783 and 1 : 947 respectively). Table II shows the results of the first *ClB* experiment: males were X-rayed and mated with *ClB* females, and the mutations induced in the treated X-chromosomes were detected in F_2 males. These experiments gave the same result as the first ones: a very pronounced acceleration of the process of mutation by X-rays. A third series of experiments was performed with the *attached-X* method: males were rayed and crossed to $\widehat{XX}Y$ females. As in the *ClB* experiments, two dosages, t_2 and t_4, were used (t_4 being twice as high as t_2). In 1490 F_1 ♂♂ from fathers treated with t_2,

61 showed visible abnormalities, some of which were mutations, identical with previously known ones; 86 F_1 ♂♂, out of 1150 from fathers treated with t_4, showed visible abnormalities.

Further tests of the induced variations showed that many of them were allelomorphic to or identical with mutations already known from the spontaneous process of mutation in *D. melanogaster*. It was found that, besides gene mutations, chromosome abnormalities and rearrangements (breaks, deletions, inversions, translocations) are also produced by X-ray treatment.

Thus, the results of Muller's experiments showed (1) that X-rays induce mutations at a very high rate, (2) that different kinds of mutations can be induced, and (3) that in general the induced process of mutation is very similar to the spontaneous. The latter is shown by the fact that in both cases the same types of mutations appear, that lethals are much more frequent than visible mutations, and that most of the induced visibles are homologous with spontaneous mutations.

Many special questions concerning the nature of the process of mutation and of X-ray action were already raised and partially answered experimentally in these first experiments of Muller. They will be discussed in later chapters.

Confirmations of Muller's results. Soon after the first publication by Muller (1927) several papers appeared confirming and partially extending his findings to other tissues or species (Hanson, 1928; Hanson and Heys, 1928; Patterson, 1928; Serebrovsky and associates, 1928; N. T.-R.[1], 1928; Weinstein, 1928; Whiting, 1928). Hanson showed that substantially the same results can be obtained with radium treatment as with X-rays. Patterson and N. T.-R. induced somatic mutations by X-ray treatment of eggs and young larvae of *D. melanogaster*. Whiting induced mutations by X-rays in the parasitic wasp *Habrobracon juglandis*.

At approximately the same time as Muller's first publications appeared, the papers of Gager and Blakeslee and of Stadler describing their X-ray and radium experiments with *Datura* (Gager and Blakeslee, 1927; papers of Blakeslee and colleagues, 1928) and with barley and maize (Stadler, 1928) were published. These experiments were started and performed independently of Muller's work and reached substantially the same conclusions: short-wave radiations induce gene mutations as well as chromosome abnormalities in the progeny of treated plants and seeds.

(2) *General validity of the effects of short-wave radiations on the process of mutation.*

Soon after the appearance of Muller's first papers the radiation work was extended to several other species and to many special questions relating to the process of mutation.

It was shown that different short-wave radiations (γ-rays of radioactive substances, X-rays of different wave-lengths, ultra-violet rays), and also free electrons (β-rays of radium, cathode rays), if properly applied, will induce all known types of heritable variations (gene mutations and different types of chromosome abnor-

[1] Throughout this article the initials N. T.-R. are printed for N. W. Timoféëff-Ressovsky.

malities). Mutations can be induced in different tissues: mature and immature sperm, mature and immature eggs, early developmental stages of the germ track, and different somatic tissues.

The most intensive and detailed work was done on *D. melanogaster*. But extensive work has also been done on other organisms (maize, barley, *Antirrhinum*, *Nicotiana*, *Habrobracon*), while with still other species the results obtained show that they will react genetically in substantially the same way. The following organisms have already been used in radiation genetic work: (*a*) Protista: *Chilodon uncinatus* (MacDougall); (*b*) plants: Mucoraceae, *Sporobolomyces*, *Nadsonia* (Nadson and Philippov), wheat (Stadler, Sapehin, Delaunay), oats (Stadler), barley (Stadler), rye (Levitsky), maize (Stadler), cotton (Horlacher, Goodspeed), vetch (Levitsky), *Crepis* (Levitsky, Navashin), *Hyacinthum* (de Mol), *Nicotiana* (Goodspeed), *Datura* (Blakeslee), tomatoes (Lindstrom), *Mirabilis* (Brittingham) and *Antirrhinum* (Stubbe); (*c*) animals: *Apotettix* (Nabours), *Habrobracon* (Whiting), *D. melanogaster* (Muller and many others), *D. funebris* (N. T.-R.), *D. virilis* (Demerec, Fujii), *D. pseudoobscura* (Schultz), and mice (Dobrovolskaia-Zavadskaia, Snell)[1].

The above-mentioned facts lead to the conclusion that short-wave radiations exert a very general effect on the germ plasm. We must expect that, if properly applied, short-wave radiations will produce genetic changes in any treated organism, and probably in any tissue capable of genetic reactions.

Treatment with short-wave radiations is an effective and sure method for accelerating the process of mutation. It has also the advantages that the dosages applied can be exactly measured and can be varied both qualitatively and quantitatively. In connection with these advantages many special problems arise within the field of radiation genetics. A part of these are purely genetic, in the sense that the treatment is merely a method for producing the variations which serve as material for genetic analysis. Other problems are connected with the analysis of the action of rays on mutability, and, thereby, of the nature of the process of mutation.

(3) *Relation between the quantity of radiation and the mutation rate.*

The first question arising in any experiment dealing with the effects of treatment is the relation between the applied dosage and the reaction obtained. The first *ClB* experiments of Muller (1928 *c*) showed that there is a direct proportionality between the dosage of X-rays applied and the percentage of mutations induced (Table II). In the following years several special experiments were performed to determine exactly the relation of the induced mutation rates to the dosages.

The first special tests on *D. melanogaster*, using the *ClB* method, were made by Hanson. He treated the males with 150 mg. radium for 9 hours and varied the thickness of the filter. His results showed that the rate of sex-linked lethals was directly and simply proportional to the ionisation rate of the dosages applied (Hanson and Heys, 1929).

[1] Recently Astaurov (1933) has published the first positive results of his extensive radiation-genetic experiments on inducing mutations in the silkworm *Bombyx mori*. And Pirocchi (1933) describes mutations induced by X-rays in *Macrosiphum rosae*.

Oliver (1930) performed a similar test using X-ray treatment. The quality of rays (50 kV. and 1 mm. aluminium) was kept constant; the dosage was varied by varying the time of exposure and was measured in r. units; dosages from 285 to 4560 r. were used. The results showed a direct, linear proportion to the dosage. Substantially the same results were obtained in experiments by Schechtman (1930) and by Efroimson (1931), using dosages from 1125 to 9000 r., and by N. T.-R. (1934), with dosages from 1200 to 4800 r. (Fig. 3).

Thus, a number of independent experiments, performed at different laboratories and using dosages from 285 to 9000 r., have shown that in *Drosophila* there exists a direct linear proportionality between the dosage (ionisation rate) of radiation and the induced mutation rate. This regularity seems to hold also for different special types of mutations: unpublished data of N. T.-R. show that with the doubling of X-ray dosage the rate of induced sex-linked visible mutations is also doubled; and

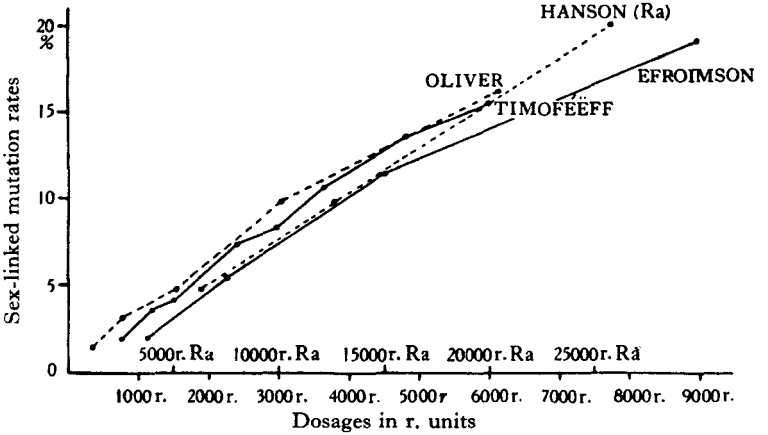

Fig. 3. The proportionality of the rates of sex-linked mutations in *D. melanogaster* to the dosages applied. X-ray experiments of N. T.-R. (1934), Schechtman (1930), and Oliver (1932), and radium experiments of Hanson and Heys (1932).

unpublished data of Muller suggest that the same is true in the case of induced breaks of the *X*-chromosome.

Stadler (1930, 1931), even before the above-mentioned *Drosophila* experiments were published, found that in barley and maize the rate of induced mutations also shows direct and probably linear proportionality to X-ray dosage.

Stubbe (1933), working on *Antirrhinum*, finds in his last publication that the rate of induced mutations rises until the X-ray dosage of 400 r. is reached, then it drops and begins to rise at a dosage of 3200 r. (the dosages used were: 100, 200, 400, 800, 1600, and 3200 r.). He gives a rather complicated theoretical explanation of this phenomenon. He assumes that low dosages of X-rays produce mutations only in certain labile genes; when higher dosages (over 400 r.) are reached, these labile genes begin to mutate to lethal allelomorphs, causing the death of mutated gametes and, correspondingly, the mutation rate drops; if still higher dosages are applied, other, stable, genes begin to mutate and the mutation rate begins to increase again.

But the experimental results of Stubbe are based on insufficient numbers of tested plants, so that even the largest differences between the rates of mutations within the range of higher dosages (400 and 1600 r.) are not statistically significant. In the light of the exact results obtained in *Drosophila*, barley and maize, this type of relationship between dosage and mutation rate seems to be improbable; and it even disagrees with the results obtained by Stubbe (1932) in his earlier experiments on *Antirrhinum*[1].

On the basis of the present results we must admit that the induced mutation rate is directly proportional to the ionisation rate of the radiation dosage and that this proportionality is probably linear (not following an S-curve)[2]. Thus, we do not expect to find a minimal active dosage of X-rays and radium, below which no mutations can be induced. And the second conclusion is that a rather simple relation must exist between the electron hits and the mutation reactions.

Table III. *Sex-linked mutations in* D. melanogaster *and equivalent radium dosages applied in different concentrations.* (*From Hanson and Heys*, 1932.)

Dosages			No. of F_2 cultures	No. of lethal mutations	Percent. of lethals
mg. Ra	Exposure in hours	Dosage in r. units			
300	0·5	6315	637	30	4·71
4	37·5	6316	636	30	4·72
2	75	6315	626	29	4·57
300	1	12630	626	61	9·75
4	75	12632	622	60	9·65
2	150	12627	619	56	9·53
4	150	25263	366	74	20·22

Another type of experiment performed on *Drosophila* gives further, indirect, evidence in favour of the above conclusions. It is known that in many physiological reactions (of more or less complex nature) to X-rays and radium the so-called "time factor" plays an important role: the effects are less pronounced if the same quantitative dosage is applied in diluted or spaced form. Such experiments, using fractioned and diluted dosages, have also been performed on induced mutation rates in *D. melanogaster*.

Patterson (1931), using the *ClB* method, applied an X-ray dosage of 1220 r.: (1) continuously (in 10 min.), and (2) divided into eight fractions (of 75 sec. each), spaced over different periods of time (intervals between the fractions being 24, 12, 8, 1 or 0·5 hours in different sets of experiments). The fractioning had no influence upon the induced mutation rate.

[1] Even if further tests should prove the reality of the phenomenon, other explanations must be taken into consideration. The physicist, Dr B. Rajewsky, proposed, for instance (in a discussion), as an explanation of Stubbe's results, the assumption that low dosages of X-rays produce some chemical changes in the tissues, which secondarily induce mutations; this chemical induction ceases when higher dosages are reached and causes the first unexpected peak on the proportionality curve.

[2] To avoid misunderstanding it must be stated that the word "linear" designates a simple relation between agent and reaction; the empirical curve will, certainly, show some "saturation effects" when high enough mutation rates are reached (because of the occasional coincidence of two or more induced mutations per gamete).

Hanson and Heys (1932) performed *ClB* experiments applying equivalent radium dosages of different concentrations. Table III shows that the dilution of the dosage had no effect on the percentages of mutations induced.

Table IV shows the results of the *ClB* experiments of N. T.-R. (unpublished),

Table IV. *Sex-linked mutations in* D. melanogaster *produced by equivalent concentrated, diluted and fractioned X-ray dosages.*

(*Timoféëff-Ressovsky, unpublished data.*)

Dosage and nature of treatment	No. of F_1–F_2 cultures	No. of sex-linked lethals	Percent. of sex-linked lethals
Controls	1827	2	0·11
3600 r.; continuous in 15 min.	493	54	10·9
3600 r.; continuous in 6 hours	521	60	11·5
3600 r.; fractioned, 6 × 5 min., every 24 hours	423	47	11·1

where equivalent X-ray dosages were applied in concentrated, diluted and fractioned form. Neither the dilution nor fractioning of the dosage had any effect on the rate of induced mutations.

The above experiments, proving the absence of an effect of the "time factor" on the induced mutation rates, again show the simple proportionality of the percentage of mutations to the ionisation rate of the dosage applied.

(4) *Quality of radiation as related to the process of mutation.*

Limits of radiation frequencies effective in producing mutations. From the shortest rays, the γ-rays of radium (Hanson and Heys, 1928, 1929 *a*; Stadler, 1928, 1930, 1931) to the softest X-rays (Efroimson, 1931; Schechtman, 1930; Stubbe, 1933), within the range of wave-length from 0·01 to 2·0 Å., all kinds of rays produce mutations in abundance.

It is much more difficult, however, to test whether ultra-violet rays are effective in producing mutations. The experiments of Altenburg (1928, 1930) on ultra-violet treatment of *D. melanogaster* gave negative or inconclusive results as did Stubbe's (1932) ultra-violet experiments on *Antirrhinum majus*[1]. In experiments of MacDougall (1929, 1931) on the infusorian *Chilodon uncinatus*, gene mutations and chromosome abnormalities were produced by ultra-violet rays. Results showing some positive effect of ultra-violet treatment on the mutability of *D. melanogaster*, although statistically insignificant, were obtained by Geigy (1931) and by Promptov (1932). The trouble is that in most cases, even in an object as small as *Drosophila*, the ultra-violet rays are absorbed in the surface tissues and do not penetrate to the gametes.

[1] In recent, still unpublished, work Stubbe has treated *Antirrhinum* pollen with ultra-violet and visible light of different wave-lengths (Noethling and Stubbe, 1934). He found a statistically significant increase of the rate of mutation following the treatment of pollen cells with ultra-violet rays of about 300 mm. wave-length. Treatment with visible light had no influence on the rate of mutation. These experiments show that ultra-violet rays are effective in inducing mutations, if suitable objects (allowing the rays to penetrate into the chromosomes) are used. I am very much obliged to Dr H. Stubbe for the permission to use his unpublished data. Recently Altenburg (*Science*, **78**, 1933) also got positive results in *Drosophila*, in treating the "germ pole" of developing fertilized eggs with ultra-violet light.

Hanson (unpublished) and N. T.-R. (1931 *a*) independently found that the chitinous tergites and a tissue layer 0·5 mm. thick of *Drosophila* absorb almost all ultra-violet rays. Further treatments of Protozoa, pollen cells and perhaps of *Drosophila* eggs and young larvae, may yield definite results, which would be of great importance because ultra-violet rays of different wave-lengths have different specific photo-chemical actions. In applying various parts of the ultra-violet spectrum we can hope to exert specifically differentiated influences on the process of mutation.

Free high-speed electrons (β-rays of radioactive substances and cathode rays), if they penetrate into the gametes, probably produce mutations in the same way as do the X-rays. The effectiveness of β-rays was proved by Hanson in *D. melanogaster* (Hanson and Heys, 1928, 1929 *a*; Hanson and Winkleman, 1929), by Gager and Blakeslee (1927) in *Datura* and by Stadler (1928, 1930, 1931) in barley and maize. N. T.-R. found in a preliminary test that a substerile dosage of cathode rays slightly raises the percentage of sex-linked lethals in *D. melanogaster*. The low effectiveness is probably due, as also in the case of ultra-violet treatments, to the poor penetration of cathode rays, most of them being absorbed before reaching the chromosomes of the gametes.

Attempts to induce mutations in *D. melanogaster* by electricity (Horlacher, 1930; Schmitt and Oliver, 1933) and by supersonic vibrations (Hersh, Karrer and Loomis, 1930) gave negative results.

Thus, the above-mentioned facts show that all kinds of ionising radiations capable of penetrating into the gametes will produce mutations in abundance. The work with ultra-violet rays, being photo-chemically of special interest, is technically difficult because of the low penetration power and the pronounced physiological actions of these rays.

Relation between the quality of rays and the mutation rate. The discovery that rays of various wave-length produce mutations brings us to the next problem: the quantitative comparison of the action of qualitatively different rays. Such experiments have been performed within the range of different X-rays.

Schechtman (1930) and Efroimson (1931), working on *D. melanogaster* with equivalent dosages of very soft (1·75 Å.) and hard (0·22 Å.) X-rays found that, if a correction for the lower penetration of the soft rays is made, equal dosages (in r. units) of soft and hard X-rays produce approximately equal percentages of sex-linked lethals.

Hanson, Heys and Stanton (1931) varied the voltage from 40 kV. to 99 kV. in their X-ray experiments on *D. melanogaster* and found that the rate of induced mutations remains proportional to the ionisation rate of the dosages applied, regardless of the wave-lengths of the rays.

Table V shows the results of *ClB* experiments on *D. melanogaster* by N. T.-R., using equivalent dosages (approx. 3600 r.) of soft (25 kV., 0·5 mm. aluminium) and hard (160 kV., 0·25 mm. copper + 3 mm. aluminium) X-rays. The percentage of induced sex-linked mutations was in both cases practically identical.

Stubbe (1933), using equivalent dosages of very soft (8–10 kV.), soft (30–70 kV.) and hard (125–175 kV.) X-rays, found no statistically significant differences in the rates of induced mutations in *Antirrhinum majus*.

In *D. melanogaster*, where very many visible mutations are induced by X-rays and radium, no qualitative differences in the mutabilities (differences in the kind of induced mutations) following treatment with different X-rays and radium can be detected.

All the above experiments show that within the range of X-rays the wave-length has no specific significance in the production of mutations. This is to be expected if the physical and photo-chemical properties of X-rays are taken into consideration. Of great importance would be the comparison of the effects on mutability of the different ultra-violet rays (having different photo-chemical actions) and of free electrons of different speeds (cathode rays at different voltages), if the technical

Table V. *Relation between quality of rays and the rate of sex-linked mutations in X-ray experiments with* D. melanogaster. *Soft and hard X-rays given in equal quantities* (3600 r.), *produce the same numbers of mutations, showing independence between mutation rate and quality of X-rays.* (*Timoféëff-Ressovsky, unpublished data.*)

Dosage	No. of cultures	No. of mutations	Percent. of mutations
Approx. 3750 r.; 25 kV., 0·5 mm. aluminium	486	63	12·9
Approx. 3750 r.; 160 kV., 0·25 mm. copper + 3 mm. aluminium	516	64	12·4
Controls	1827	2	0·11

difficulties caused by the low penetration power of these rays could be surmounted (using suitable objects, *e.g.* plant pollen or Protozoa, or suitable developmental stages of higher animals[1]). Also an exact comparison of the mutation rates, induced by equivalent dosages of γ-rays and X-rays is still wanted[2].

(5) *Various conditions which might have an influence on the induced process of mutation.*

In the preceding chapters experiments were reviewed in which the quality or the quantity of the radiation applied was varied, all other conditions being kept constant. Now we will analyse various other conditions, which could have an influence upon the mutability induced by radiations.

Stability of different genes. The first question arising is whether or not different genes are equally susceptible to radiation. From our findings as to the spontaneous mutability of *D. melanogaster*, we know that different genes certainly have different mutation rates, and, consequently, different degrees of stability as regards those factors which produce the "spontaneous" mutations (Morgan, Bridges, Sturtevant, 1925; Muller, 1923). The rates of spontaneous mutations of different genes vary from 1 to more than 50 in the several millions of *D. melanogaster* flies analysed.

[1] See footnote on p. 422.

[2] Dr A. Pickhan, working at this laboratory and using exactly comparable and equivalent dosages of γ-rays of radium and of X-rays, found no difference in the mutation-inducing power of these rays (unpublished).

Similar conditions have been found in other well-analysed forms, as *Antirrhinum* (Baur, 1924) or maize (Stadler, 1930 *b*).

The X-ray work with *Drosophila*, *Antirrhinum* and maize shows that the frequency of induced changes is also different for different genes. In general, in *D. melanogaster* most of the frequent spontaneous mutations are also frequently induced by X-rays (Muller, 1928 *d*, 1930 *a*; N. T.-R., 1931 *a*). But probably exceptions will be found to this rule, as is already the case in maize (Stadler, 1930 *b*).

Different allelomorphs of the same gene may also show different degrees of stability in X-ray experiments. Within the white-eye series of allelomorphs in *D. melanogaster* the darker allelomorphs mutate more frequently under the influence of the same X-ray dosage than do the lighter allelomorphs (N. T.-R., 1932 *c*, 1933 *b*). Fig. 4 depicts the mutability of two different "normal" allelomorphs of this series, showing pronounced differences in the total frequency of mutations and in the relative frequencies of different mutational steps induced by X-ray treatment (N. T.-R., 1932 *a*, 1933 *b*).

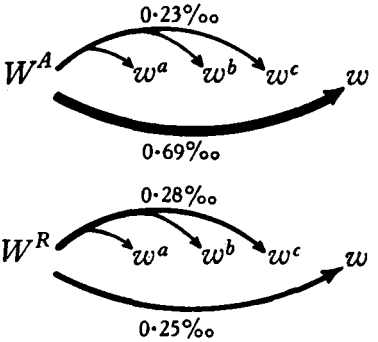

Fig. 4. Differences in the mutation rates from two different "normal" allelomorphs (W^A and W^R) of the white-eye series of *D. melanogaster*, to intermediate allelomorphs (w^a, w^b, w^c) and to white (w), induced by the same X-ray dosage (4800 r.).

Mutability in different species, races and individuals. The fact that different genes and even different allelomorphs of the same series have different stabilities makes it rather difficult to compare the mutabilities induced in different species, races or individuals by the same X-ray dosage. It is hard to say whether the differences obtained are due to some specific, racial or individual factors of a general kind, or simply to the fact that one of the groups contains a number of more or less stable genes or allelomorphs as compared with the other group.

The only clear and convincing results in this field were obtained by Stadler on X-ray induced mutation rates in related plant species showing polyploid series of chromosome numbers. He worked on four species of oats and on four species of wheat. *Avena brevis* and *A. strigosa* have a haploid number of chromosomes (7). The same number in *A. byzantina* and *A. sativa* is 21, showing that they have probably a triploid set of chromosomes. The haploid chromosome number is 7 in *Triticum monococcum*, 14 in *T. dicoccum* and *T. durum*, and 21 in *T. vulgare*, showing also a polyploid series. Mutations could be induced in a high rate in

Avena brevis, *A. strigosa*, and *Triticum monococcum*, the species with the simple set of chromosomes. In *T. dicoccum* and *T. durum*, the species with double sets of chromosomes, the induced rates of mutations were much lower, and in *Avena byzantina*, *A. sativa*, and *Triticum vulgare* (having 21 chromosomes) no mutations were induced at all (Stadler, 1929). These results of Stadler are shown in Table VI and can be interpreted in the following way: in polyploid species most of the genes are present in double (or triple) number, so that most of the recessive mutations cannot manifest themselves even in homozygous condition, as they are covered by the normal allelomorphs present in the other homologous chromosomes.

In experiments of H. A. and N. W. Timoféeff-Ressovsky more sex-linked mutations were induced in *D. melanogaster* than in *D. funebris* (by treatment with the same X-ray dosage). But at least a part of this difference is due to the more exact method of detection of sex-linked lethals in *D. melanogaster* (H. A. Timoféeff-Ressovsky, 1930 *a*, *b*; N. T.-R., 1931 *a*). In comparing sex-linked mutation rates (or in general, the mutation rates of certain single chromosomes) in different

Table VI. *Relation between the induced mutation rates and chromosome numbers in related species showing polyploid series* (*oats and wheat*). (*From Stadler*, 1929.)

Species	Haploid number of chromosome	No. of cultures	No. of mutations	Mutations per 1 r. unit $\times 10^{-6}$
Avena brevis	7	394	5	4·1
A. strigosa	7	1116	9	2·6
A. byzantina	21	337	0	0
A. sativa	21	413	0	0
Triticum monococcum	7	133	4	10·4
T. dicoccum	14	107	1	2·0
T. durum	14	444	6	1·9
T. vulgare	21	745	0	0

species the possible differences in relative "genetic" sizes (number of genes contained) of these chromosomes must also be taken into consideration.

No exactly analysed cases of differences in the induced mutation rates between races or individuals within a species are known which are not due to the presence of single frequently mutating genes. Serebrovsky found that in *D. melanogaster* the number of mutations obtained from different X-rayed individuals varies according to chance distribution, thus showing that there are probably no especially "mutable" individuals in this species (Serebrovsky *et al.*, 1928). The same was proved to be true by N. T.-R. (unpublished data).

From cases where different mutation rates are induced by the same dosage in different species or races, conclusions as to the different degrees of susceptibility of these groups to X-rays must be drawn very carefully. From Stadler's results with polyploid species we have already seen that the doubling of the set of chromosomes can mask the detection of mutations. In *Drosophila* we know many mutations suppressing other mutant characters ("specific suppressors," Bridges); and it is evident that races homozygous for suppressors of relatively frequently mutating

genes will not show these mutations, typical for other, related, races or species. Besides the *karyotypic masking*, due to the doubling of a part or of the whole set of chromosomes, and the *genotypic masking*, due to specific suppressors, we must reckon with the possibility of *phenotypic masking* of mutations in related species or races. The latter is due to the epistatic covering of certain mutant characters (making them undetectable) by some already present mutant characters. These theoretical considerations are mentioned in order to show at least a part of the difficulties which will be encountered in comparing the mutability of different species. Much further work must still be done in this direction.

Induced mutability in different sexes and tissues, and under different physiological conditions. In all tissues and cells so far tested, in which mutations can be detected, it has been found that radiation treatment induces mutations. Here we will mention some tests in which an exact comparison of the induced mutation rates in different tissues and under different conditions was carried out.

Muller (1929) found that in *D. melanogaster* the same dosage of X-rays induces

Table VII. *Number of sex-linked lethals in sperm which was in different developmental stages at the time of the X-raying of males. The* P_1 ♂♂ *were X-rayed, mated with ClB* ♀♀ *and then every* 5 *days they were mated to new virgin ClB* ♀♀. (*From Timoféëff-Ressovsky*, 1930 *d*.)

Age of sperm in days after X-raying	No. of fertile F_2 cultures	No. of sex-linked lethals	Percent. of lethals
1–5	417	29	6·9
5–10	491	41	8·3
10–15	481	35	7·3
15–20	478	19	4·0
20–25	411	13	3·1
25–30	389	7	1·8
Controls	984	0	0

more mutations in mature sperm than in the various developmental stages of the eggs.

The *Drosophila* males, after they have hatched from the pupa, already contain a certain number of mature sperm cells. When these are used up in the first copulations they are replaced by fresh ones, developing from the immature germ cells present in the gonads. Thus in an adult male, different developmental stages of the germ cells are rayed during the treatment. If treated males are mated every 4, 5 or 7 days to fresh virgin females, then in the first broods sperm will be used which has been X-rayed in the mature stage; and in the successive broods, sperm X-rayed in different immature developmental stages fertilises the eggs. Such experiments (using the *ClB* method) were independently performed by Harris, Hanson and Heys, and N. T.-R., and all gave the same results: the percentage of mutations decreases in subsequent broods, *i.e.* sperm X-rayed in mature stages contain more mutations than the sperm immature at the time of treatment (Hanson and Heys, 1929 *b*; Harris, 1929; N. T.-R., 1930 *d*). Table VII shows the results obtained by N. T.-R.

These results can be interpreted in two ways: (1) that the immature germ cells are much less susceptible to X-rays and the genes in them are much more stable, or (2) that at least a part of the difference in the induced mutation rates is due to germinal selection, since we were dealing with sex-linked lethals in male germ cells (having only one *X*-chromosome). It is known that genes are inactive in mature sperm of *Drosophila*, but they could influence the cell life and division rate in immature germ cells. N. T.-R. (1930 *d*, 1931 *b*) advocated the second interpretation, since he found, contrary to the results of Hanson, that the rate of non-lethal visible sex-linked mutations did not show any decrease in subsequent broods. The mortality in larval and pupal stages, caused by factors which probably do not directly influence the cell life, is equal after X-raying mature and immature sperm; but the mortality in the egg stage is much higher when mature sperm was rayed, probably because many of the induced factors influencing the early developmental stages are already underlying germinal selection if immature germ cells are rayed. A direct proof was furnished by Sidoroff (1931), who found that the percentage of autosomal lethals induced in the II-chromosome of *D. melanogaster* (which do not undergo germinal selection because the autosomes are present in diploid number in the immature germ cells) remains practically constant in all subsequent broods. But autosomal translocations are induced more frequently in mature sperm, as was shown by Schapiro (1931).

Another question to be solved was whether the process of the origin of mutations is bound up with some stages of chromosome division. The fact that mutations are frequently induced in dormant seeds (Stadler, 1930 *a*) and in mature sperm seems to disprove this assumption. But it may be admitted that even in the mature sperm the chromosomes are not dormant and undergo, slowly, some preparations for further division. In this case, and if the process of the origin of mutations is confined to some of these stages of the chromosome cycle, we should expect to get different mutation rates by treatment of young and old mature sperm in *Drosophila*. Experiments of Harris (1929) and some other data show that the mature sperm already present in the young males is not being absorbed or ejaculated if they are not allowed to copulate. Thus, some of the males can be rayed just after they hatch from the pupae and others can be kept isolated for 20–25 days and then rayed. Such tests were made by N. T.-R. (1931 *b*) and gave no statistically significant difference between the mutation rates induced in young and old sperm by the same X-ray dosage (Table VIII). Thus, even if the chromosomes of mature sperm are not "dormant," the origin of mutations is not restricted to some stage of chromosome division, since the frequency of this particular stage should be different in young and old sperm.

As was already stated, mutations can be induced in somatic tissues, giving rise to individuals showing mosaic distribution of the characters in question (Patterson, 1929 *a*, *b*; Stadler, 1930; N. T.-R., 1929 *c*). Patterson described the induction of somatic mutations (by X-raying eggs and larvae) in various tissues of *D. melanogaster*. But exact data on the rate of somatic mutations are present only for the sex-linked white-eye locus in *D. melanogaster* (Patterson, 1929 *a*). In Patterson's experiments

the rate of somatic white mutations was about 1 : 9000; the frequency of white mutations induced by the same dosage in mature sperm is at least twice as high. Thus, the rate of mutation of definite single genes can be different in different tissues.

That some physiological conditions can exert an influence upon the induced mutation rate in a definite tissue was shown by the fact that Stadler (1928, 1930 *a*), applying the same X-ray dosage to dormant and to germinating seeds of barley, got many more mutations from the treatment of germinating seeds. Recent experiments of Hanson show that in *Drosophila* the rate of induced mutations may be influenced to some extent by starvation of the flies before or by anaesthesia during the X-ray or radium treatment (Hanson and Heys, 1933 *a*, 1933 *b*).

Relation between the induced mutability and various other agents combined with X-ray treatment. Agents other than short-wave rays can be applied in combination

Table VIII. *Rate of sex-linked lethals, produced by X-rays in fresh and in old mature sperm cells of* D. melanogaster. (*From Timoféëff-Ressovsky*, 1931 *b*.)

Types of cultures	No. of fertile F_2 cultures	No. of sex-linked lethals	Percent. lethals
Controls	984	0	0
Young males X-rayed and mated just after treatment	718	82	11·4
Old males, held 20–25 days without females, X-rayed and mated just after treatment	539	57	10·6

with X-ray treatment to test whether they influence the rate of mutation induced by X-rays. Two such agents have already been tested: (1) impregnation with salts of heavy elements, and (2) temperature.

Stadler (1928 *b*) showed that the impregnation of barley seeds with salts of heavy metals (barium nitrate, lead nitrate, and especially uranium nitrate) increased the effectiveness of X-ray irradiation, the rate of mutation being about 1 : 16 in impregnated and about 1 : 35 in non-impregnated seeds. The chemical treatment alone induces no mutations. The findings can be explained by the assumption that the impregnated seeds absorb more X-rays than do the chemically untreated ones.

Stadler (1928 *c*, 1930 *a*) on barley and Muller (1930) and N. T.-R. (unpublished data) on *Drosophila* performed X-ray treatments at different temperatures. Stadler X-rayed barley seeds at temperatures of 10, 20, 30, 40 and 50° C., and found no effect of temperature (applied during the X-ray treatment) on the rate of induced mutations. Muller X-rayed *Drosophila* males at 8 and 34° C.; N. T.-R. did the same at 10 and at 35° C. In both cases no effect of temperature could be detected. These results are of considerable importance, showing that the process of mutation induced by irradiation is probably based on reactions of the monomolecular type, which do not follow the Van't Hoff rule.

Further work in this direction, using other accompanying agents and, if possible, mutation rates of single genes, would be of great interest.

Direct or indirect action of short-wave rays on mutability. The simple proportionality of the induced mutation rates to dosages and the results of the experiments reviewed in the preceding paragraph suggest that the action of short-wave rays on the genes is rather a direct one, understanding "direct" as the immediate local action of the primary quanta or secondary released electrons. But *a priori* it does not seem improbable that at least a part of the genetic effects of X-rays are due to some more or less stable chemical changes primarily induced in the irradiated chromosome material or even in the cytoplasm. In this case a delayed "after-

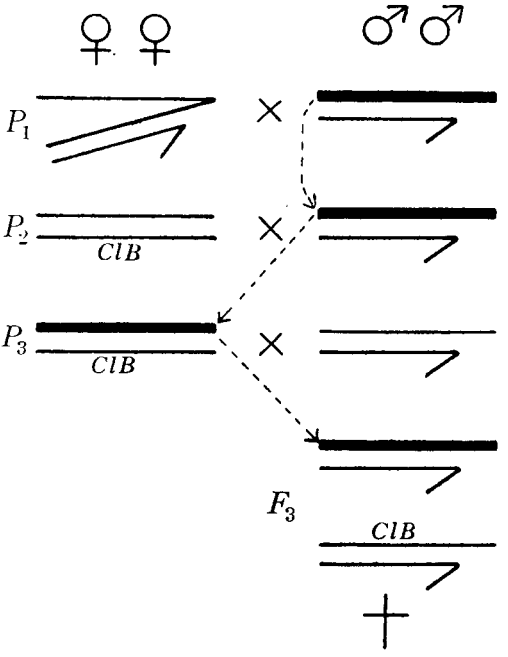

Fig. 5. Scheme of crossings made to test whether there is some "after-effect" of X-raying upon mutability in the next generations. P ♂♂ are rayed and crossed to *attached-X* ♀♀; all F_1 ♂♂ surviving and showing no new mutations contain an X-rayed X-chromosome in which no mutations arose during the treatment. These males are crossed to *ClB* ♀♀ and in F_3 all mutations arising in the previously treated X-chromosome can be detected. The treated chromosomes are represented by darker lines.

effect" would be detectable, increasing the rate of mutation in previously rayed chromosomes and in untreated chromosomes crossed into an X-rayed cytoplasm.

These latter assumptions can be tested experimentally in *D. melanogaster*. Fig. 5 shows the method of crossing suitable for the detection of an eventual "after-effect" of X-ray treatment on the rate of mutation. Normal F_1 ♂♂ from crosses of X-rayed ♂♂ to *attached X* ♀♀ contain an X-rayed X-chromosome in which no mutations arose immediately during the treatment; the males are then crossed to *ClB* ♀♀ and in F_2 from these crossings (*i.e.* in F_3 from the beginning of the experiment) the rate of mutation in the previously treated X-chromosomes can be determined. Such, or similar, experiments were independently performed by

Muller (1928 *c*, 1930 *a*), N. T.-R. (1930 *c*, 1931 *b*), and Grüneberg (1931), and all gave the same results: no "after-effect" of X-ray treatment on the rate of mutation could be detected.

Fig. 6 shows the method of crossing used by N. T.-R. (1931 *a*, *b*) in experiments destined to test if there is any influence of X-rayed cytoplasm on the rate of mutation in untreated *X*-chromosomes. Untreated males are crossed to X-rayed *attached-X* females; the F_1 ♂♂ from these crossings contain an untreated *X*-chromosome in rayed cytoplasm; the rate of mutation in their *X*-chromosomes is tested by further *ClB* crossings. These experiments showed that X-rayed cytoplasm has no effect at all upon the rate of mutation in untreated chromosomes.

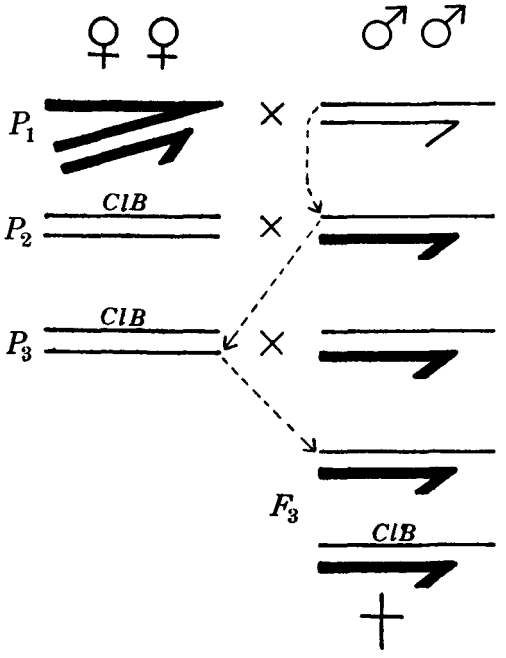

Fig. 6. Scheme of crossings made to test whether the X-raying of the egg plasm has an influence on the origin of mutations in untreated chromosomes. *Attached-X* ♀♀ are rayed and crossed to untreated ♂♂; the F_1 ♂♂ thus contain untreated *X*-chromosomes in X-rayed plasm; they are crossed to *ClB* ♀♀ and in F_3 the percentage of mutations arising in the untreated *X*-chromosomes within the treated plasm can be determined. The treated chromosomes are represented by darker lines.

The results of the experiments of N. T.-R. (1930 *c*, 1931 *a*, *b*) on the "after-effect" of X-rays and on the influence of X-rayed cytoplasm upon the rate of mutation in untreated chromosomes are shown in Table IX.

Thus, all the above experiments, as well as the indirect evidence mentioned at the beginning of this section, tend to deny the existence of an "indirect" effect of X-ray treatment upon mutability. On the other hand, there are some facts which could be explained by the assumption of an "after-effect." Muller (1928 *d*, 1930 *a*) found that X-raying of mature *Drosophila* sperm induces a certain amount of "fractional mutations," *i.e.* half-to-half mosaics, showing the mutant character

only in one-half of their body. This can be explained either by an "after-effect" of the treatment (assuming that the delayed mutation occurs just after fertilisation, in the first cleavage stages), or by the assumption that a part of the mature sperm contains chromosomes that are already split, and that in this case the mutation arises only in one-half of a split chromosome. Muller himself tends to admit the latter explanation, since some unpublished cytological observations of Shiwago show that double strand chromosomes (already split for further divisions) are likely to occur even in the resting stages of the cells.

Table IX. *The frequency of sex-linked mutations in* D. melanogaster: (1) *in untreated controls*; (2) *in cultures, containing a previously X-rayed X-chromosome, which was free of mutations just after treatment*; (3) *in cultures, containing not-treated X-chromosomes in X-rayed egg plasma*; *and* (4) *in cultures with directly treated X-chromosomes. (From Timoféëff-Ressovsky, 1931 b.)*

Types of cultures	No. of fertile cultures	No. of sex-linked lethals	No. of sex-linked visible mutations
Untreated controls	793	1	—
Cultures with previously X-rayed *X*-chromosome	756	2	—
Cultures with untreated *X*-chromosomes in X-rayed egg plasm	581	—	—
Cultures with directly X-rayed *X*-chromosomes	844	89	8

(6) *Types of induced mutations and comparison of induced and spontaneous processes of mutation.*

Classification of heritable variations. All the different types of heritable variations must be divided into two groups: plasmatical changes (variations of the "plasmotype") and genotypical changes (variations of the "genotype"). We naturally exclude all so-called "combinations," due to hybridisation and not accompanied by real changes in the original germ plasm.

Our knowledge of *plasmatical changes* is still very scanty. But they probably involve the following three types: (1) changes in some structural elements of the cytoplasm (*e.g.* plastids in plant cells), (2) adaptation of the plasmotype to changed genotypical constitution (*e.g.* in some species hybrids in plants), (3) enduring modifications or *Dauermodifikationen* (*i.e.* induced, slowly reverting, changes in the plasmatic constitution).

The *genotypical changes*, or mutations, involving qualitative or quantitative changes in the set of genes (localised in the chromosomes), can be classified according to the unit of change: it can involve either a single gene, or (without changing the genes) a chromosome, or (without changing the single chromosomes) the set of chromosomes (the "karyotype"). Thus we can distinguish three types of mutations: (1) gene mutations (changes in the single genes, leading to the formation of new allelomorphs), (2) chromosome mutations (intrachromosomal rearrangements and

quantitative changes, the genes remaining unchanged), (3) karyotype mutations (changes in number of chromosomes, genes and chromosomes remaining unchanged). Gene mutations are changes in the gene structure leading to the origin of new allelomorphs of the genes in question. Mutant allelomorphs may behave as dominants, intermediates, or recessives; they can affect any organ or characteristic of the organism; they can exert a lethal or sublethal effect, lower the viability of the organism, leave it unaffected, or (in rare cases) even raise the viability of the mutant type; single mutant allelomorphs can produce visible effects in one or few characteristics of the organism, or affect several different characters ("pleiotropic genes"). The phenotypic manifestation of the mutant allelomorphs may be full and constant, or it can be variable and even dependent upon other factors (low penetrance, variable expressivity and specificity of the genes, N. T.-R., 1931 *c*). Chromosome mutations consist in breaks, deletions or section inversions within single chromosomes and in translocations of pieces within the same or to another chromosome. Various chromosome mutations are shown in Fig. 7. Karyotype mutations consist in changes of chromosome number, the structure of chromosomes being unchanged; addition of one or more whole sets of chromosomes results in polyploidy, and the addition or subtraction of one or several single chromosomes leads to trisomics or heteroploidy. Karyotype mutations are shown in Fig. 8.

Thus, the following classification of heritable variations may be given:

A. Plasmatical changes.
   (1) Changes in structural elements of the cytoplasm (Correns, 1909).
   (2) Adaptation of the cytoplasm to changed genotype (Michaelis, 1933).
   (3) Enduring modifications (*Dauermodifikation*, Jollos, 1913).

B. Genotypical changes.
   (1) Gene mutations (mutation *sensu stricto*).
   (2) Chromosome mutations.
      (*a*) Breaks and fragmentations.
      (*b*) Deficiencies and deletions.
      (*c*) Inversions.
      (*d*) Simple translocations and duplications (intra- and interchromosomal).
      (*e*) Mutual translocations.
   (3) Karyotype mutations.
      (*a*) Trisomics.
      (*b*) Heteroploids.
      (*c*) Polyploids.

Types of induced heritable changes. All types of heritable changes mentioned in the above classification were already known from the spontaneous process of heritable variability in *Drosophila* and in some plants. All these types have appeared in the progeny of X-ray or radium treated plants and animals; but, and this is a

very important fact, no new types, unknown from the spontaneous process of mutability, have ever been found in radiation genetical work.

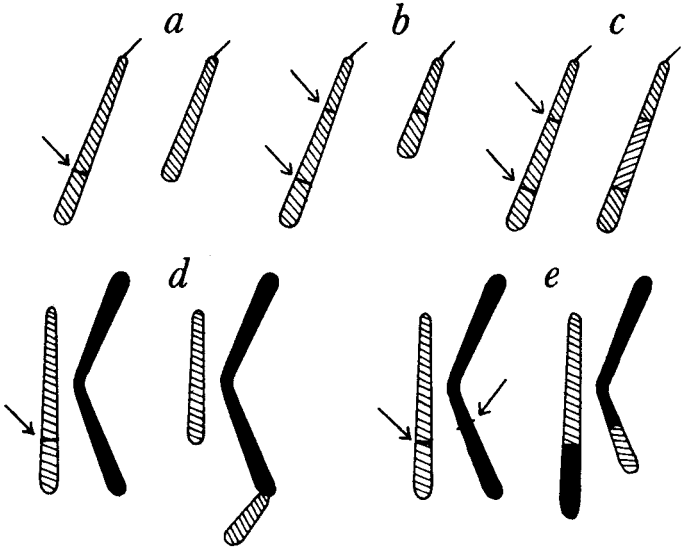

Fig. 7. Different types of chromosome mutations (chromosome breakage): *a*, simple break, followed by the loss of a part of the chromosome; *b*, deletion, following a double break; *c*, inversion of a section of the chromosome; *d*, simple translocation of a piece of one chromosome to another; *e*, mutual translocation. At the left is shown the normal and at the right the resulting mutant condition. Arrows indicate the points of breakage.

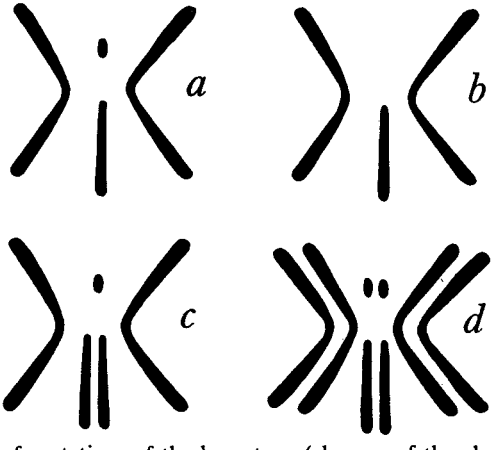

Fig. 8. Different types of mutations of the karyotype (changes of the chromosome number): *a*, a normal haploid (*n*) set of chromosomes; *b–c*, heteroploidy ($n-1$ or $n+1$, in general form, $n-m$ or $n+m$); *d*, polyploidy ($2\times n$, in general form, $m\times n$).

(1) *Gene mutations.* In the X-ray and radium work, different kinds of gene mutations were induced in *Drosophila* and other tested animals and plants. In a number of species most of the induced mutations are recessive; the same is the case in the spontaneous process of mutation in these species. But dominants are

also induced at approximately the same rate (in relation to recessives) as they occur spontaneously. The induced mutations, as well as the spontaneous, can affect all parts and characteristics of the organism (Morgan, Bridges and Sturtevant, 1925; Muller, 1928 *d*, 1930 *b*). Besides morphological characters they can also affect different physiological characteristics of the organism, and its viability (N. T.-R., 1933 *d*). Gene mutations can be induced in somatic cells and produce mosaic patterns (Patterson, 1929 *a*, *b*; N. T.-R., 1929 *c*). Mutations can be induced not only from the "normal" allelomorphs of the wild type to "mutant" allelomorphs, but also from recessive mutants back to or towards the original normal

Table X. *Reversions of recessive gene mutations in the X- and III-chromosomes of* D. melanogaster *produced by X-rays (dosages* 3600 *and* 4800 *r.). (From Timoféëff-Ressovsky,* 1933 *e.)*

X-rayed allelomorphs and their loci	Number of analysed X-rayed chromosomes containing the loci	Type and number of reversions
X-chromosome, 0 *y*	11,781	—
,, 0+*sc*^{1}	17,676	3 *sc*^{1}→*Sc*
,, 2 *w*	29,233	1 *w* →*w*^{a}
,, 2 *w*	29,233	1 *w* →*w*^{b}
,, 2 *w*^{a}	23,472	1 *w*^{a}→*w*^{b}
,, 2 *w*^{a}	23,472	1 *w*^{a}→*W*
,, 7 *ec*	17,676	—
,, 16 *cv*	6,354	1 *cv*→*Cv*
,, 25 *ct*^{6}	12,914	—
,, 40 *v*	19,268	1 *v* →*V*
,, 51 *g*^{2}	12,914	—
,, 62 *f*	24,695	5 *f* →*F*
III-chromosome, 0 *ru*	16,936	—
,, 26 *h*	16,936	1 *h* →*H*
,, 42 *th*	5,681	—
,, 44 *st*	16,936	—
,, 48 *p*^{p}	11,255	2 *p*^{p}→*P*
,, 50 *cu*	5,681	—
,, 58 *ss*	11,255	—
,, 62 *sr*	5,681	—
,, 71 *e*^{s}	16,936	1 *e*^{s}→*E*
,, 101 *ca*	5,681	—
Total	288,961	18
Controls	139,234	0

allelomorphs (Patterson and Muller, 1930; N. T.-R., 1925, 1928 *a*, 1929 *a*, 1930 *a*, *b*, 1932 *c*, 1933 *a*, *b*, *c*). Table X shows the results of X-ray experiments on induction of such reverse gene mutations. The occurrence of reverse mutations is of great importance, showing that the process of mutation does not consist merely in a destruction of the normal wild-type allelomorphs. The different kinds of gene mutations were induced not only in *D. melanogaster*, but also in all other organisms which were studied extensively: *D. funebris* (H. A. Timoféëff-Ressovsky, 1930), *D. pseudo-obscura* (Schultz, 1933), *Habrobracon* (Whiting, 1929), maize, barley (Stadler, 1931), *Antirrhinum* (Stubbe, 1932, 1933).

(2) *Chromosome mutations.* In *D. melanogaster* all types of chromosome mutations (breaks, deletions, inversions, translocations) have been induced by X-ray

treatment. Heavy dosages produce them in such abundance that even certain types needed for some special cytogenetical work can be induced by will if large enough numbers of flies are treated. This opens a wide field for cytogenetic research (Dobzhansky, 1929 *b*, 1930 *b*, 1931, 1932; Muller and Altenburg, 1930; Muller and Painter, 1929, 1932; Painter, 1931; Painter and Muller, 1929, 1932). Fig. 9 shows an X-ray induced translocation from the II to the III chromosome in *D. melanogaster*, which was tested both genetically and cytologically. Chromosome mutations can also be induced in somatic cells (Patterson, 1929 *b*, 1930 *a*). Chromosome mutations have also frequently been induced in a number of plant species:

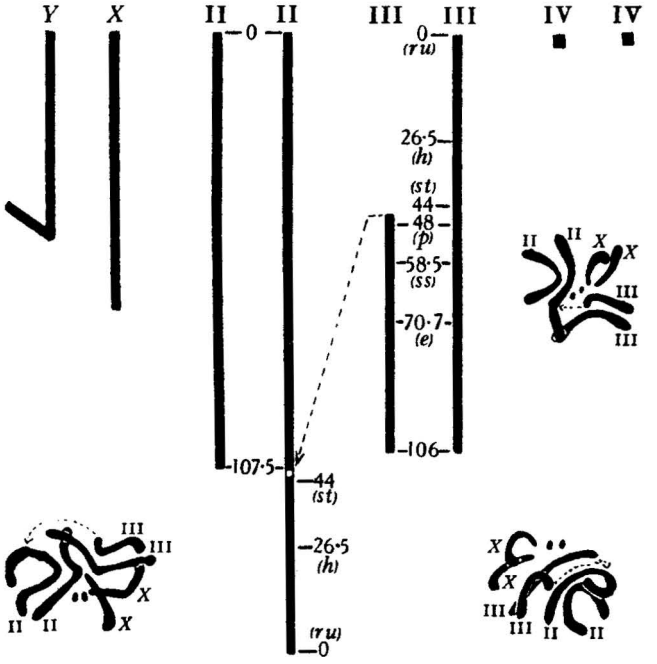

Fig. 9. An X-ray induced translocation of a large piece of the left arm of the III-chromosome to the right end of the II-chromosome in *D. melanogaster*, proved genetically and cytologically. (From Painter and Muller, 1929.)

Datura (Blakeslee, Avery, Bergner, Buchholz, Carteledge, Satina, 1928–9), *Nicotiana* (Goodspeed, 1930, 1931, 1932), maize (Stadler, 1931; McClintock, 1931), *Triticum* (Delaunay, 1930), *Secale*, *Vicia*, *Crepis* (Levitsky and Araratian, 1931; Navashin 1931).

(3) *Karyotype mutations.* Non-disjunction of the sex-chromosomes, leading to heteroploidy, was the first heritable change produced by X-rays in *D. melanogaster* (Mavor, 1921, 1922, 1924). Since then heteroploids and polyploids were frequently induced, especially in some plant species (Blakeslee, *et al.* 1928–30; Goodspeed, 1931; Levitsky and Araratian, 1931).

(4) *Plasmatic changes.* To this group perhaps belong some of the so-called "types," described by Stubbe (1932) in his X-ray work on *Antirrhinum*. These

abnormalities show inheritance only through the female, suggesting that they are caused by some factors localised in the cytoplasm. In *D. melanogaster* Woskressensky (1929) induced, by X-ray treatment, an acceleration of the time of development, which persisted for several generations and gradually disappeared. He designed this case as *Dauermodifikation*. The present author found in his X-ray experiments several variations in *D. melanogaster* and *D. funebris*, which were transmitted only by the females and gradually disappeared within three or four generations (N. T.-R., 1932 *b*). These cases do not fit any chromosomal or genotypic explanation and can best be explained as being due to plasmatic enduring modifications. But, as was already mentioned in the preceding section, our knowledge of the role of the cytoplasm in inheritance is still very meagre, and much further exact work must be done in this direction.

Comparison of the induced and spontaneous processes of mutation. It has already been stated above that no new types of "X-ray or radium mutations" were observed in any of the treated species, and that there is a far-going general parallelism between the induced and spontaneous processes of mutation.

A more or less detailed comparison can only be made in *D. melanogaster*, a species in which we already know several hundred different spontaneous and induced mutations. This comparison shows that most of the induced gene mutations are identical with, or allelomorphic to, already known spontaneous mutations. Some of the induced gene mutations are quite new; but every year new spontaneous mutations are also found. Both in the spontaneous and induced processes of mutation we observe about the same proportions of recessives and dominants, and of visibles and lethals, the latter being more than ten times as frequent as the non-lethals. Most of the recurrent spontaneous mutations have also been observed more than once in both X-ray and radium experiments; and such mutations as, *e.g. Bar*, which arose (spontaneously) only once in a very large number of flies, have not yet been induced by X-rays or radium. This means that in general (although many exceptions from this rule will probably be found) the same genes behave as stable or less stable ones in both the spontaneous and induced processes of mutation (see Table 33 in N. T.-R., 1931 *a*). As to the chromosome mutations, it has already been stated that all of these, induced in abundance in X-ray or radium experiments, belong to one of the types already known from spontaneous mutation. Here, however, there seems to be a difference: the relative frequency of chromosome mutations (as compared with gene mutations) is probably higher in the induced process.

Another *Drosophila* species, *D. funebris*, differs in the general type of its spontaneous mutation from *D. melanogaster* (it has more semi-dominant mutations, relatively more mutations with variable phenotypic manifestation, and some of the most common mutations of *D. melanogaster*, such as *white* or *yellow*, have never appeared in *D. funebris*), but the same general differences have been found in its X-ray induced mutability (H. A. Timoféëff-Ressovsky, 1930 *a*, 1930 *b*).

In most of the other species used in X-ray work the comparison has not yet been carried through in detail. But even if some specific differences are found, the

general similarity of the induced and spontaneous process of mutation is large enough to allow far-going deductions from the analytical radiation genetic work to the spontaneous mutability in different species.

(7) *The nature of the effect of rays on the process of mutation.*

From the results of the radiation genetic experiments reviewed above, some conclusions can be drawn upon the kind of action exerted by the short-wave rays on the process of mutation. The most important of these conclusions are the following.

(1) The action of the short-wave radiations upon the germ plasm is of a general kind. All hitherto known types of genetic changes can be produced by ionising radiation if the latter reaches the cells in question without killing the treated organism.

(2) Within the range of X-rays and radium-rays the genetic action of radiation seems to be non-specific: if equivalent dosages are used it is independent of the wave-length applied. Within the range, at least, of (soft and hard) X-rays the action is also quantitatively independent of wave-length, being simply proportional to the amount (the ionisation rate) of the dosage. A more or less specific action is only to be expected within the ultra-violet rays, since different parts of their spectrum have different photo-chemical actions. The shortest γ-rays can perhaps produce an effect somewhat different from that of X-rays, because the basic physical action on atoms of the former is probably somewhat different from that of the latter (ejection not only of electrons but also of positrons)[1].

(3) The action of short-wave radiation upon genes and chromosomes is a direct and simple one. This follows from the absence of a genetic "after-effect" of X-ray treatment, independence of the X-ray induced mutation rate from temperature (applied during X-ray treatment), absence of an influence of the "time factor" (dilution or fractioning of the dosage) upon the rate of induced mutations and from the simple direct proportionality of the induced mutation rates to the dosages. If mutations were produced, not directly by the radiation quanta or the released electrons, but indirectly by some physiological or chemical reactions primarily induced by radiation, then we should expect to find some of the above-stated complications in the relation between radiation treatment and induced mutation rate.

(4) Radiation is capable of producing mutations in different tissues, under different physiological conditions, and in the presence of different accompanying factors. But at least some of these secondary conditions and factors (*e.g.* rate of metabolism, impregnation with salts of heavy metals, etc.) can influence the rate of mutations induced by radiation treatment. This shows that the process of mutation is dependent not only upon the physico-chemical structure of the mutating units (genes or chromosomes), but also upon the nature of the chemical environment of these units.

(5) The different types of induced gene mutations show that the genetic action of short-wave rays is not merely destructive, but rather reconstructive, since

[1] See footnote 2 on p. 424.

"direct" and "reverse" mutations (*i.e.* mutations of the same gene in opposite directions) can be induced by irradiation. The absolute frequency of mutations is dependent upon the dosages, but the relative frequencies of different gene changes, and the direction of mutations, is determined by the structure of the genes in question.

(6) From the results of different radiation genetic experiments the following statements can be made concerning the nature of gene mutations and the structure of the gene. (*a*) The fact that reverse gene mutations (Table X) can be induced by irradiation shows that in general mutations are not merely losses of previously present genes. In several cases (Patterson and Muller, 1930; N. T.-R., 1930 *b*, 1932 *c*) "direct" and "reverse" mutations were induced by X-rays directly one from another in *D. melanogaster*. The following cases are examples. From a normal allelomorph of the white series eosin was induced, and the latter, under further treatment, produced a reversion back to normal. A spontaneously arisen eosin gave, under treatment, a reversion to normal, and this normal mutated under further treatment back to eosin. Mutations were produced by X-rays from normal to forked and from this forked back to normal, and from forked to normal and from this normal back to forked. Mutations were produced from pink to normal and from this normal back to pink. From these cases it becomes evident that the action of X-rays cannot be of a purely destructive kind and that the gene mutations, at least in these and in similar cases, cannot be simple losses of the previously present gene, or even of a specific part of the gene substance, because as Muller has expressed it, it is highly improbable that "if with one blow we punch the gene out, with the next we would punch it in again." The most plausible assumption would be, then, that gene mutations are reconstructions of the gene, *i.e.* some physico-chemical changes of its structure (N. T.-R., 1932 *c*). (*b*) The absolute frequency of gene mutations is determined by the dosage. But different genes, and even different allelomorphs of the same gene, give distinctly different relative mutation rates, thus showing that the structure of the gene is the main cause of its relative stability. In no case was it possible to find a dependence of the kind of induced mutations on the kind or the amount of radiation applied; this shows that the structure of the gene must also be responsible for the direction of its mutability. The X-ray induced mutability at the locus of white in *D. melanogaster* shows (Fig. 12) some characters of "determinate variation," the different mutational steps being not unordered but occurring with different specific frequencies (N. T.-R., 1930 *b*, 1932 *c*, 1933 *b*, *c*). This must also be determined by some specific characteristics of the gene structure. Thus we come to the conclusion that the structure of the genes of a given group of organisms determines to some extent the evolutionary potencies and the direction of evolution of this group. (*c*) Concerning the physico-chemical nature of the genes, two views can be confronted. The genes are either fixed quantities of specialised matter (consisting of several or even many equal physico-chemical units), or they are physico-chemical units (molecules, micellae, or colloid particles of specific structure). The former view is expressed and elaborated in Goldschmidt's quantitative theory of gene action and gene mutation (1928). This view, and Goldschmidt's theory in its relation to gene structure, is merely a specification of Bateson's "presence or

absence" hypothesis, and its difficulties in explaining many facts in our present knowledge about allelomorphic differences and mutation in different directions are just the same as those of the classical form of the "presence or absence" theory. The facts of reverse mutation, determinate mutability of some of the genes (N. T.-R., 1932 *c*, 1933 *b*, *c*), frequently mutating genes (Demerec, 1928, 1929), and of "non-serial" allelomorphic series (Dubinin, 1929, 1932; N. T.-R., 1932 *c*, 1933 *c*), are much easier to fit into the second view, *i.e.* that genes are physico-chemical units and gene mutations are changes in the structure (and, in consequence in the properties) of these units (Patterson and Muller, 1930; N. T.-R., 1930 *b*, 1932 *c*, 1933 *b*, *c*). This view may serve as a working hypothesis; our present empirical knowledge is far too insufficient to build up more detailed theories of the structure of the gene. But radiation genetics gives us new methods for attacking the gene problem.

(8) *Applications and conclusions.*

Applications of radiation genetic methods. Besides their own lines of development (*i.e.* the analysis of the action of short-wave rays upon the germplasm), some of the methods of radiation genetics can already be applied to the study of various general genetic questions. In some of these questions really exact work can only be done through the experimental induction of mutations by X-rays or radium. The methods of radiation genetics have already been applied with success in the following cases.

(1) *Induction of chromosome mutations.* One of the fields of application of X-ray induced chromosome mutations is cytogenetics and especially the elaboration of "cytological chromosome maps." An exact comparison of "genetical" and "cytological" map distances was first made by Muller and Painter (1929) and by Dobzhansky (1929). Now, through special investigations of Dobzhansky (1929 *b*, 1930 *a*, *b*, 1931, 1932) and of Muller and Painter (Muller and Painter, 1932; Painter, 1931; Painter and Muller, 1932), we are already in possession of preliminary "cytological maps" of the three long chromosomes of *D. melanogaster*. A comparison of the genetic and cytological maps of the *X*-, II- and III-chromosomes is shown in Fig. 10. Induced inversions, deletions, fragmentations and translocations of chromosomes are also used in studies on crossing-over and chromosome conjugation and disjunction, both in *Drosophila* and in maize.

Another new and important field of research connected with X-ray induced chromosome mutations is the study of the action of varying amounts of single individual genes. Although some work in this direction had already begun before the discovery of the genetic effects of X-rays (Bridges, Mohr, Stern), an effective and general attack on this problem is connected with experimental induction of chromosome mutations *en masse*. The first experiments dealing with this question were made by Muller (1932 *b*), who studied the effects of different individual genes in hyperploid combinations, using small fragments of chromosomes (containing the gene in question) induced by X-rays. Muller classifies mutations on their counter-action on the original allelomorph from which they arose. He distinguishes the following chief types of mutations: (*a*) hypomorphs, (*b*) hypermorphs, (*c*) antimorphs, (*d*) neomorphs, and (*e*) amorphs. The hypomorphs are mutations

producing the same, but less pronounced, effect as those allelomorphs from which they arose; if present in supernumerary condition, they approach the effect produced by the original allelomorphs from which they arose. Hypermorphs produce a stronger, but similar, effect as the original allelomorphs. Antimorphs are those mutant allelomorphs which produce an effect opposite to that of the original allelomorph: the phenotypic end-effect of different antimorphic combinations is the result of their antagonistic actions. Neomorphs produce an effect which is "new" for this gene: the original allelomorph is an "amorph" in respect to the

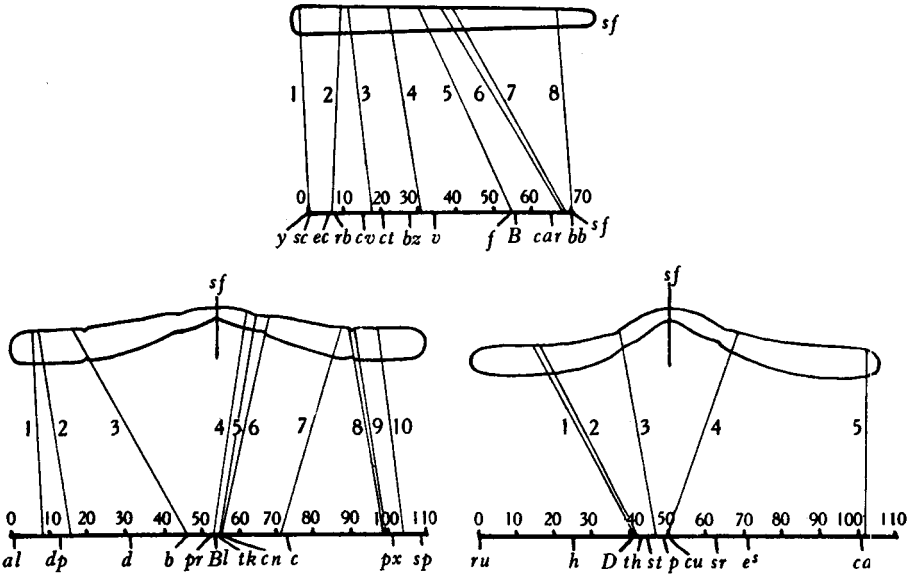

Fig. 10. A comparison of the genetic (crossing-over data) and cytological (cytological analysis of translocations from and to genetically known loci) maps of the *X*-chromosome (Muller and Painter, 1932), II-chromosome (Dobzhansky, 1930) and III-chromosome (Dobzhansky, 1929) of *D. melanogaster*. The lines 1, 2, 3, etc., connect the points of breaks on the genetic map and on the actual chromosome, showing, in both cases, identity of gene sequence but different relative distances between the genes.

character or characters produced by the neomorph and does not affect its development at all. Muller's classification of mutations, and further work in this direction (made possible by the application of radiation genetics), will bring us a step forward in our knowledge of the structure, nature and action of genes.

(2) *Somatic mutations as an embryological method.* In Section IV (6) it was mentioned that somatic mutations can be induced by X-raying fertilised eggs and larvae at various stages (Patterson, 1928, 1929; N. T.-R., 1929 *a*, *b*, *c*). Patterson has shown that X-raying *D. melanogaster* larvae at different stages of development produces somatic eye-colour mutations, resulting in eye mosaics with mutant areas of different size. The mutant areas are large if embryos or young larvae are rayed, and they are small if the larvae were older at the time of treatment. The reduction of the size of mutant areas with the raising of the age of the larvae at the time of treatment is shown in Fig. 11. Thus, the production of somatic mutations, especially

the use of the relatively frequent somatic chromosome mutations (in special genetic combinations, making them phenotypically detectable), can be used as an analytical method in embryology, allowing the study of the growth and differentiation of some of the *Organanlagen*. The production of somatic mosaics may also be of interest in connection with the question of interrelations between tissues of different genetic constitution (as studied by Sturtevant, 1932).

(3) *Comparison of the mutabilities in different species and races.* A really effective attack on this question can only be made with the help of radiation genetic methods. In comparing mutation rates in different species we must reckon with certain difficulties, especially with the "masking effects" already mentioned in Section

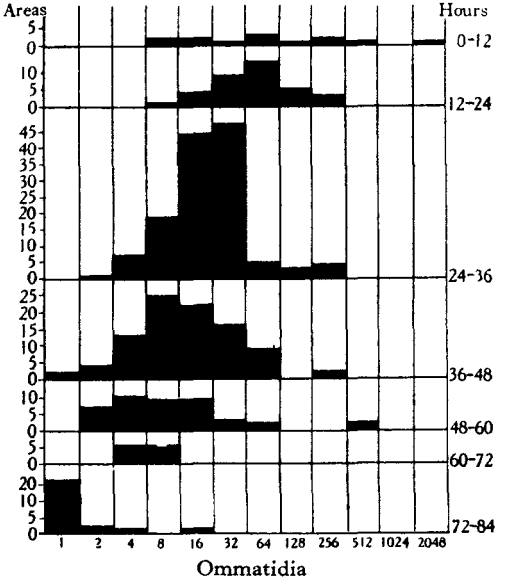

Fig. 11. The relation between the age (in hours) at which larvae of *D. melanogaster* were X-rayed and the number of ommatidia in the mutant eye spots (induced as somatic mutations of the white locus). (From Patterson, 1929.)

IV (5). But, if we take into account the different possible sources of errors, irradiation treatment can be used with success as a method in comparative genetics.

(4) *The study of the mutability of single individual genes.* X-ray and radium treatment is so far the only effective way of studying the mutational potencies of single individual genes.

In *D. melanogaster* the mutability of three sex-linked loci has been studied with the help of X-ray treatment. Dubinin, Serebrovsky, and their collaborators have studied mutations at the locus of scute which affect different groups of bristles and hairs on the head and thorax. Several dozen mutations were already induced at this locus and the phenotypic effects of different scute allelomorphs were compared. On the basis of these studies, the "theory of step-allelomorphs" was developed (Dubinin, 1929, 1930, 1932; Serebrovsky and Dubinin, 1930). The essential point of this hypothesis is the assumption of a complex structure of the genes. Different

parts ("subgenes" or "centres") of the gene affect different groups of bristles, and the various allelomorphs are mutations of different single "centres" or of neighbouring groups of "centres" of the same original gene. It is not the right place here to discuss the whole evidence for and against this hypothesis; in any case the induction of mutations at the locus of scute yields us a very interesting and important material for work upon the properties and structure of the genes.

Patterson and Muller (1930) and N. T.-R. (1932 *c*, 1933 *a*) induced many mutations by X-rays at the locus of forked in *D. melanogaster*. In this locus "direct" mutations (from the normal allelomorph to, or towards, forked) and "reversions" (from forked to, or towards, normal) are induced with about the same frequencies, showing the potential reversibility of at least some of the mutational changes,

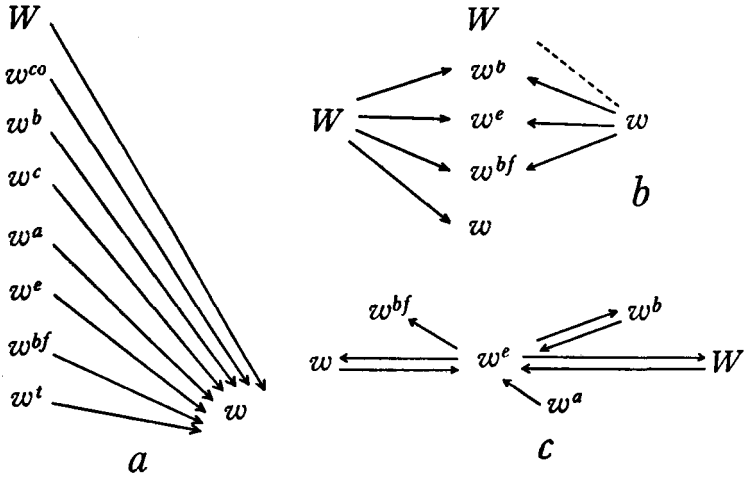

Fig. 12. X-ray induced mutations at the locus of white in *D. melanogaster*. *a*, The white allelomorph was induced from all other tested allelomorphs of this series; *b*, the allelomorphs blood, eosin and buff were induced as direct mutations from normal and as reverse mutations from white; *c*, all induced mutations from and to eosin.

being thus a serious objection against a generalisation of the "presence or absence" theory.

Special experiments have been performed on a large scale to study the mutability at the locus of white eye in *D. melanogaster* (N. T.-R., 1930 *b*, 1932 *a*, *c*, 1933 *b*, *c*). The rates of different mutational steps, induced by X-raying (with a constant heavy X-ray dose), were compared in flies containing different allelomorphs of this gene. As a result it was found that quite different mutations can be induced at this locus: "direct" and "reverse" mutations, mutations from one particular allelomorph to various others, and mutations from various allelomorphs to a particular one (Fig. 12). But the rates of the different mutations are different, some of the mutational steps being frequent and others very rare, thus showing a certain modal direction or "determinate variation" of the mutability at this locus. The results of these experiments are shown in Table XI. Another feature of these experiments was the finding of two otherwise indistinguishable normal allelomorphs of the white series,

differing both in the frequency and the modal direction of their mutability (N. T.-R., 1932 *a*, *c*, 1933 *b*, *c*). The different mutabilities of these two allelomorphs are shown in Fig. 3.

All the above experiments show that, in any case, the methods of radiation genetics can be applied with success to quantitative studies of the variability of individual genes. One of the most interesting problems arising in this connection is the experimental study of the evolution of the genes. We know that new allelomorphs can arise by mutation, and we must admit that, in the course of the evolution of a species, the number of the genes, and the profound properties of some of them (*e.g.* their general phenotypical effects and the direction of modal mutability) must also undergo changes. The Bar-eye mutation in *D. melanogaster* is probably a case in which a new gene arose in this species (Sturtevant, 1925). Some facts from the above-cited comparison of different scute mutations suggest that the genes scute and achaeta perhaps represent a stage in the differentiation of one original

Table XI. *Comparison of different mutation rates within the* white *eye series of multiple allelomorphs in* D. melanogaster, *produced by X-ray treatment* (*dosage* 4800 *r.*). (*From Timoféeff-Ressovsky*, 1933 *e.*)

Mutations	No. of flies	No. of mutations	Rates of mutations in $^0/_{00} \pm m$	Differences of rates in $^0/_{00} \pm m$ diff.
All direct	129,000	62	$0{\cdot}481 \pm 0{\cdot}061$	$0{\cdot}436 \pm 0{\cdot}063$
All reverse	134,500	6	$0{\cdot}045 \pm 0{\cdot}018$	
$w^e \rightarrow w^{-e}$	39,000	15	$0{\cdot}385 \pm 0{\cdot}111$	$0{\cdot}308 \pm 0{\cdot}119$
$w^e \rightarrow w^{+e}$	39,000	3	$0{\cdot}077 \pm 0{\cdot}044$	
$W \rightarrow w^x$	48,500	37	$0{\cdot}763 \pm 0{\cdot}125$	$0{\cdot}708 \pm 0{\cdot}128$
$w \rightarrow w^x$	54,000	3	$0{\cdot}055 \pm 0{\cdot}032$	
$W \rightarrow w$	48,500	25	$0{\cdot}515 \pm 0{\cdot}102$	$0{\cdot}254 \pm 0{\cdot}116$
$w^x \rightarrow w$	80,500	21	$0{\cdot}261 \pm 0{\cdot}056$	
$W \rightarrow w^x$	48,500	37	$0{\cdot}763 \pm 0{\cdot}125$	$0{\cdot}393 \pm 0{\cdot}143$
$w^{e-co} \rightarrow w^x$	73,000	27	$0{\cdot}370 \pm 0{\cdot}071$	$0{\cdot}305 \pm 0{\cdot}078$
$w^{-bf} \rightarrow w^x$	61,500	4	$0{\cdot}065 \pm 0{\cdot}032$	

gene into two. And the finding of two normal allelomorphs of the white-eye series differing in degree of stability and modal direction of their mutabilities, indicates a differentiation of a gene (within the population of a species) in respect to its profound fundamental properties. The results of further experimental work in this direction will be of great interest, devoted to the search for allelomorphs (of the same gene) differing in direction and relative frequencies of mutation, and in the kind of characters affected by these mutations.

(5) *Practical applications*. The methods of radiation genetics can be practically applied in plant breeding. Most of the new mutations lower the viability of the organism and thus are, in most cases, of negative biological and economical value. But in certain combinations with other mutations, and in the presence of certain modifiers, even such mutations can restitute the normal viability of the wild type (N. T.-R., 1933 *d*), and thus have practical significance in plant breeding. The production of mutations *en masse* by X-rays or radium will have a special practical significance in those cases in which selection has already reached its limit, and in which crossing with related races or species must, for some reason, be avoided.

However, the practical significance of the induction of gene mutations is minimised by the fact that in the living populations of our crop plants and domesticated animals and of their wild relatives we have tremendous funds of still unutilised genes, which can be used in husbandry. But the induction of chromosome mutations will probably have unlimited applications, allowing of the "construction" of quite new karyotypes[1]) in our cultivated plants.

In man radiation genetics has a purely negative significance: we must avoid any X-ray or radium treatments of the gonads (not leading to continuous sterility) in order not to accelerate the funds of injurious mutations already present in a rather high percentage in human (especially in European) populations. I believe slight treatments applied to many persons, performed without the control of good specialists, and without considering the danger of genetic injuries, to be most harmful in this respect. We must not forget that in *Drosophila* a general mutation rate of 1 per cent. (*i.e.* 1 mutation per 100 gametes) is produced by X-ray dosages of about 40–50 r. units[2]).

Conclusions. The following statements can be made concerning problems already solved in radiation genetics: (1) The genetic action of short-wave rays is a general one, capable of inducing all known types of mutations in all hitherto adequately tested objects. (2) The induced process of mutability shows far-going similarity and parallelism with the spontaneous one. (3) The induced rate of mutations is directly proportional to the dosage applied. (4) Within the range of X-rays the wave-length (if equal dosages are applied) has no specific influence upon the rate or the kind of induced mutations.

The following questions are not yet solved, or not yet decided with sufficient exactness: (1) The genetic action of ultra-violet rays; this question is of special interest since, theoretically, different ultra-violet rays could exert specific influences upon the germplasm. (2) An exact comparison of the influences of equivalent dosages of X- and γ-rays upon the process of mutation[3]. (3) The role of different accompanying factors and of different physiological conditions in the induction of mutations by short-wave radiations. (4) The intimate physical nature of the genetic action of radiations. (5) Various special genetic problems connected with the induction of gene and chromosome mutations (*e.g.* quantitative studies of mutability in different species, studies on the direction of mutability and on "evolutionary potencies" of single individual genes, studies on the mechanism of chromosome mutation, etc.).

Soon after the first discovery of a pronounced action of X-rays on the rate of mutation, Muller himself and several other biologists expressed the idea that the origin of spontaneous mutations could perhaps be ascribed to "natural radiations"

[1] The "karyotype" is the number and form of the chromosomes typical of a given species (Levitsky, 1924).

[2] The calculation of the dosage producing *a general mutation rate* of 1 per cent. is based on the following data: 3000 r. produce about 10–15 per cent. sex-linked mutations; the genetically active part of the *X*-chromosome constitutes about one-fifth to one-sixth of the whole set of chromosomes in *D. melanogaster*, and the mutability of the autosomes is as intensive as that of the *X*-chromosome; the general rate of mutations produced by 3000 r. is, accordingly, about 60–75 per cent.; a mutation rate of 1 per cent. is thus produced by 40–50 r. units.

[3] See footnote 2 on page 424.

present in the environment (Muller, 1928; Babcock and Collins, 1929; Hanson and Heys, 1930; Joly and Dixon, 1929; Olson and Lewis, 1928; N. T.-R., 1929; Tschetverikov, 1929). But calculations, carried out independently by Muller and Mott-Smith (1930), N. T.-R. (1931 *a*), and Efroimson (1931), show that the amount of "natural radiation" is insufficient to account for the rate of spontaneous mutations. Muller and Mott-Smith estimated that the actual amount of natural radiation is 1333 times too low, N. T.-R. estimated that it is 462 times too low, to produce the observed rate of spontaneous mutations. I mentioned the possibility (N. T.-R., 1929 *d*) that the concentration of radioactive substances in the living organisms, discovered by Vernadsky (1929, 1930), could account for at least some of the spontaneous mutations. But it is clear that the small amounts of radioactive substances contained in living matter cannot account for all spontaneous mutations. We must thus search for other sources of factors inducing mutation, within the organisms and in the environment.

V. HEAT AND OTHER TREATMENTS AS CAUSES OF MUTATION.

Most of the genetic experiments hitherto performed on the production of mutations with agents other than X-rays or radium either do not fulfil the requirements detailed in Section III, or they have given doubtful results. Many of them will therefore be omitted, or only mentioned briefly, below.

(1) *Temperature experiments.*

We will omit a discussion of older work and concentrate our attention on modern genetic experiments using temperature as agent for inducing mutations. The whole problem can be divided into two distinct fields of research, connected with two different methods of treatment: (1) the study of the influence upon the rate of mutation of different temperatures lying within the "normal physiological temperature scale" for the given organism, and (2) experiments with "temperature-shocks," *i.e.* treatment for a short time with extreme temperatures, having a sub-lethal or substerilising action.

Experiments within the range of normal temperatures. As early as 1919 Muller, after having found methods of determining the normal rate of spontaneous mutations in *Drosophila*, published the results of his first temperature experiments (Muller and Altenburg, 1919). Flies kept at higher gave somewhat more mutations than flies kept at lower temperatures. But this result was inconclusive, the difference in the rates of mutation being statistically insignificant. Further experiments, published 1928, gave substantially the same results (Muller, 1928 *b*): flies reared at 27° C. showed about three times as many mutations as those kept in 19° C. Unpublished experiments of N. T.-R. (1927–30) confirmed the results obtained by Muller: at 25° C., the flies gave about three times as many mutations as at 15° C. Taken all together they give a statistically quite significant and conclusive result; the spontaneous rate of mutation is directly proportional to the temperature, and the rate of mutation is tripled by an increase of temperature of about 10° C. In other words, the spontaneous rate of mutation follows the Van't Hoff rule.

This latter conclusion, suggesting that the spontaneous rate of mutation behaves as an ordinary multimolecular chemical reaction, is of great interest in connection with the results of radiation genetic experiments. One of the general conclusions which can be drawn from radiation genetics is that mutation belongs to the type of monomolecular reactions, not following the Van't Hoff rule. Experiments of Muller, Stadler and N. T.-R. have shown that different temperatures applied during irradiation have no influence upon the induced rate of mutation (Section IV (5)), thus leading to the same conclusion that mutations are monomolecular reactions. Most of the X-ray induced mutations are identical with spontaneous ones; and the same individual mutational event (the change of a definite allelomorph, leading to the formation of another definite allelomorph) cannot possibly be in one case a monomolecular and in another a multimolecular reaction. Thus there seems to be a discrepancy between the results of radiation and temperature experiments. But this discrepancy disappears if we assume that in the temperature experiments certain sources of mutation-inducing factors, and not the mechanism of the mutation event itself (which is monomolecular) follow the Van't Hoff rule. At the end of the preceding section (IV (8)) we had already reached the conclusion that some internal sources of mutation-inducing factors must exist. We do not know what they are; perhaps some processes of chemoluminescence (subject to the Van't Hoff rule) take place within the organism, and so constitute factors inducing mutation. At present any theorising in this direction is useless; only further experimental work will show whether this or some similar hypothesis conforms with the empirical facts.

Experiments with temperature shocks. This method consists in treating the organisms with "substerilising dosages" of temperatures (high or low), lying beyond the limits of normal physiological conditions.

In *D. melanogaster* this method of treatment was first applied by Muller (1928 *d*). He treated adult males with substerilising dosages of 36° C. (40–60 hours) and mated them to *ClB* ♀♀. The treated series gave a slight, statistically insignificant, increase of lethal mutations. Similar experiments (adult ♂♂ treated at 37° C. for a period of 20 hours) were made independently, and at the same time (1927–8), by N. T.-R. (unpublished); they gave substantially the same results. The same treatment was applied on a large scale by Muller and Mackensen in 1932 (exhibited at the Sixth Intern. Congr. Genet.) and also gave only a very slight increase of the rate of lethal mutations. The results of these experiments are summarised in Table XII.

In the preceding experiments adult males were treated. N. T.-R. also applied the same treatment (1927–8, unpublished) to old larvae: 5–6 days old larvae were subjected to a temperature of 37° C. for about 15 hours, and the hatching males were mated to *ClB* ♀♀. These experiments gave negative results: the rate of lethal mutations showed no significant increase. Efroimson (1932), using a similar method of treatment, got a slight, but statistically significant increase of the rate of lethal mutations in the treated series.

Goldschmidt (1929) treated *D. melanogaster* larvae (5 days old) with 37° C.

(12 hours). The hatching flies were crossed *inter se* (in most cases in mass cultures) and inbred for three generations. In some of the treated series many visible mutations (affecting eye and body colour, wings, bristles, etc.) were found in F_1 and F_2. The most striking finding in these experiments was the fact that almost all the mutations appearing in F_1 and F_2 were recessives, and a part of them even autosomal recessives. If contamination and segregation of mutations already present in heterozygous condition in the original cultures are excluded, this finding can only be explained by the assumption of a very pronounced specific action of the agent upon certain genes. An autosomal recessive mutation must be induced *en masse* in order to have a chance of appearing in F_2 or even in F_1. This is the conclusion drawn by the author from his experiments (Goldschmidt, 1929). Substantially the same method of treatment was used by Jollos (1930, 1931 *a*, *b*, 1932) in his experiments on induction of mutations in *D. melanogaster*. Jollos drew the conclusion that temperature shocks, if applied to subsequent generations, induce (in

Table XII. *Experiments on the effect of heat treatment of adult males of* D. melanogaster *on the rate of sex-linked mutations.* (*Muller*, 1928 *d*, 1930 *a and unpublished data of Muller and Timoféëff-Ressovsky.*)

Author and date of experiments	Analysed chromosome	Treatment	Series	No. of cultures	No. of mutations	Percent. of mutations	Diff.
Muller, 1928	*X*-chromosome (*ClB* method)	W series: adult ♂♂ treated with 35–36° C. for 24–40 hours. C series: untreated controls	W	493	4	0·81	1·2
			C	482	2	0·41	
Mackensen and Muller, 1932			W	5952	24	0·40	3·4
			C	5887	10	0·17	
N. T.-R. 1927–28		W adult ♂♂ in 37° C. for about 20 hours. C untreated controls	W	758	3	0·39	0·8
			C	617	1	0·16	

certain genes) "determinate mutation," proceeding step by step from the normal allelomorph towards the extreme mutant allelomorph. In his last paper (Jollos, 1933) he describes the results of some of his experiments showing that slight differences in the method of treatment (moist or dry heat) cause different specific effects on mutability (specific induction of different mutations). He thus draws the conclusion that the process of mutation can be directed by will, using slight modifications of the heat treatment.

The experiments of Goldschmidt and Jollos raise a number of most interesting questions. But, unfortunately, they do not solve them. In these experiments the most important question, namely that of the quantitative mutation-inducing action of heat treatment, was not adequately analysed. Moreover, all similar experiments performed in other laboratories have given results which are quite different from those of Jollos. Experiments by Rokizky (1930), Ferry (Ferry, Shapiro and Sidoroff, 1930), Redfield and Schultz (1931, demonstrated at the Sixth Intern. Congr. Genet. 1932), Demerec (*ibid.*), Sturtevant (*ibid.*) and Plough (Plough and Ives,

1932) are summarised in Table XIII. None of them have given results similar to those of Goldschmidt and Jollos. A slight increase in the rate of mutation, following heat treatment, was obtained by Plough; but, although he treated eight subsequent generations of flies, no mutation *en masse* or "determinate mutation" of certain genes could be observed. Similar results have recently been obtained by Grossman and Smith (1933) and in unpublished experiments of N. T.-R.; in the latter experiments the *attached-X* method of crossing was used and an exact determination of the rate of sex-linked mutations was made.

Thus it seems that in *Drosophila* the genetic effect of temperature shocks is non-specific and not at all so pronounced as the effects of radiation treatment.

Table XIII. *Experiments performed to test whether heat treatment* (37° C.) *of larvae* (3–6 *days old, for* 12–24 *hours in heat*) *increases the rate of mutations in* D. melanogaster. (*From exhibits at the Sixth Intern. Congr. Genet.*)

Authors and date of experiments	Treatment	Generations	Series	No. of cultures	No. of flies	No. of mutations
Rokitzky, 1930	After Goldschmidt	F_1–F_3	Treated	—	15,147	5
			Control	—	2,731	1
Ferry, Shapiro and Sidoroff, 1930	After Goldschmidt	F_1–F_2	Treated	265	11,771	0
			Control	62	2,590	0
Redfield and Schultz, 1931	After Goldschmidt	F_1–F_2	Treated	359	38,025	5
			Control	62	27,379	2
Demerec, 1931	After Goldschmidt	F_1–F_2	Treated	215	33,305	1
			Control	37	8,176	0
Sturtevant, 1932	3–5 days old larvae several hours in 37° C. or intermittent in 37° and in 4–10° C.	F_1–F_2	Treated	—	39,098	2
			Control	—	—	—
Plough, 1932	6 days old larvae for 24 hours in 37° C.	F_1–F_8	Treated	580	110,000	18
			Control	236	55,000	2

In plants, treatment with temperature shocks was applied to *Antirrhinum* by Baur (1930) and Stubbe (1930, 1932). These experiments gave negative results.

(2) *Experiments with other agencies.*

Almost all experiments in which agents other than radiation or temperature are used involve special difficulties. In most cases we do not know whether the treatment reaches the germ cells, and, if so, in what form it reaches them.

Chemical treatments. We will not here discuss the older literature, since it does not fulfil the requirements of exact experimentation. More recently Harrison and Garrett (1926) reported that melanistic mutations were produced *en masse* in butterflies (*Selenia*) by lead and manganese added to the food. But certain recent experiments (Lycklama and Nijeholt, 1932), and the extraordinary high mutation rate obtained by Harrison and Garrett, together with some theoretical considerations

(the high concentration of melanistic mutations in wild populations), render it improbable that all of these mutations were really induced by the treatment. Further experiments, using carefully selected and inbred material, must in any case be made before the question of chemical induction of mutations in butterflies can be decided.

Experiments on the induction of mutations in mice by alcohol treatment have been performed in many different laboratories. But these experiments have not yet yielded any conclusive results. Even the authors who believe that mutations can be induced by alcohol, and at the same time perform exact experiments on a large scale (*e.g.* Bluhm, 1930), cannot prove the existence of hereditary effects of alcohol treatment in an objective and conclusive way.

Extensive and exact experiments have already been performed with *Drosophila*, using different chemical treatments as mutation-inducing agents. Morgan tried the effects of ether and alcohol (1914) with negative results. Mann made extensive experiments on the effects of alcohol, arsenic, quinine, morphine, methylene blue, lead, lithium and copper as agents for inducing mutations. All these experiments gave negative results (Mann, 1923). Muller treated *D. melanogaster* with semilethal concentrations of lead acetate (1 per cent. of the food), arsenic trioxide (0·015 per cent. of the food) and manganese chloride (0·62 per cent. of the food) throughout the whole life cycle (Muller, 1928 *d*, 1929, 1930 *a*). None of these agents showed any influence on the rate of mutation. Muller also made experiments using Janus green (0·25 per cent. of the food, throughout the whole life cycle). These experiments, performed on a large scale (1058 F_1–F_2 *ClB* cultures from treated males and 1013 untreated control cultures) also gave negative results (Muller, 1930 *a*). Ssacharoff (1932, 1933) has recently published his results on treating *D. melanogaster* eggs with iodine in potassium iodide (for 2 min.). The treated series gave rather more mutations than the controls, but the difference is statistically insignificant.

Thus, all experiments hitherto performed on chemical treatments of *Drosophila* have given negative, or (as in the case of iodine treatment) inconclusive, results. Much further work must be done to find out whether chemical treatments exert an influence upon mutation in *Drosophila*. The most interesting feature of such experiments would be the discovery of a specific, differential, action of a certain agent upon certain definite genes. And the chief difficulty of these experiments will be the discovery of methods which would enable the agents applied to penetrate into the chromosomes of the gametes of the treated organisms.

The chemical treatment of plants is technically easier. Here the method of chemical treatment of the seeds can easily be applied, the latter being a physiologically rather resistant stage of the plant. The seeds can either be treated with solutions or they can even be centrifuged in these solutions, in order to accelerate the process of penetration of the chemicals.

Exact experiments on production of mutations in plants by chemical treatment were first made by Stadler (1928 *b*). He found that the impregnation of barley seeds with salts of heavy metals (barium, lead and uranium nitrates) has no

influence on the rate of mutation. Baur (1930, 1932) and Stubbe (1930) treated seeds and seedlings of *Antirrhinum majus* with various chemicals (such as salts of heavy metals, alcohols and acids), applying the method of centrifugation in some of the experiments. The results were inconclusive, although the percentage of mutations is perhaps somewhat higher than in the controls. The methodological mistake made in these experiments is the simultaneous treatment with several dozen different agents; it is quite evident that under these circumstances every single series yields statistically insignificant results.

Other treatments. Morgan (1930) performed extensive experiments to test whether a heat injury to the eyes of *D. melanogaster* is inherited, or, at least, has some effect on the rate of mutation. This treatment is followed by a permanent, deep red, colouring of the Malpighian tubules of the treated flies. Such flies, and also untreated controls from the same stocks, were inbred. These breeding experiments gave negative results; no inheritance of this acquired character, no specific eye defects, and no detectable increase of the general spontaneous rate of mutation, was found in the progeny of treated flies. These experiments were repeated by Muller (1930 *a*); he used a method of crossing suitable for the detection of induced lethals. The results of Muller's test were also negative.

It has already been mentioned that *Drosophila* experiments have been made to test the influence of supersonic waves and of electricity on the rate of mutation. Hersh, Karrer and Loomis (1930) found (as a result of experiments performed on a very large scale) that sublethal doses of supersonic waves do not influence the rate of mutation. Experiments of Horlacher (1930) and of Schmitt and Oliver (1933), carried out independently and using somewhat different methods of treatment, showed that electricity has no influence on the rate of mutation in *Drosophila*.

VI. GENERAL CONCLUSIONS.

The above review of all experiments hitherto performed on the induction of mutations leads to the following conclusions.

The treatment with short-wave radiations and high-speed electrons (X-rays, γ-rays, β-radiation) is so far the only effective method of inducing mutations, giving constant and measurable results. It is almost certain that radiation treatment is capable of inducing hereditary changes in all organisms, since quite different plants and animals hitherto tested have given substantially the same positive results. The power of X-rays and radium to induce all known types of heritable variations makes the application of the radiation genetic methods most valuable for analytical genetic studies, for instance, in comparative genetics of related species, in quantitative studies of the mutabilities in different species and of different individual genes, in cytogenetics, in "genetic engineering" (*i.e.* in the synthesis of new genotypes and races).

We have good reasons to believe that, besides those genetic problems which have already either been solved or attacked by radiation genetic methods, in the future the solution of the most fundamental problems concerning the nature of the genes and of gene changes will be connected with radiation genetics.

All other treatments hitherto applied have given no definite nor conclusive results. Nevertheless, temperature experiments and some of the chemical treatments show that further experimentation will yield important results.

One of the most interesting future problems is the discovery of methods of treatment which will work differentially and enable us to induce at will certain types or groups of mutations. But only experiments, using thoroughly prepared, genetically pure, material, and adequate methods of treatment and breeding, will bring us further towards the solution of these important biological problems.

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