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ON GENETIC EFFECTS PRODUCED BY IRRADIATED FOOD AND FOOD COMPONENTS

by

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On Genetic Effects Produced by Irradiated Food and Food Components.

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1. Introduction.

An attempt has been made to summarize and evaluate available data on genetical effects (mutation and chromosomal aberration) induced in different organisms exposed to irradiated food or food components. Since published as well as unpublished work has been included the present report cannot be considered to be complete.

Experiments with plants and lower organisms are included because it may be stated generally that a substance which releases gene mutation in one organism is also mutagenic in all other organisms. If therefore an irradiated system (such as food or food component) is found mutagenic in e.g. higher plants, this effect should be expected to have a counterpart in mammals including man. A lower mutagenic efficiency in mammals of a principle active in microorganisms, plants, and lower animals may partly be due to unavailability of the gonads to compounds absorbed via the digestive tract or the lungs.

Positive and negative results have been obtained in different experiments even with the same organism. It is clear that enough data are not yet available for the evaluation of genetical hazards connected with human consumption of irradiated food, and the purpose of the present scientific report is not to do such an evaluation but to present the background for the further experiments needed.

2. UV effects.

It was shown relatively early that UV-irradiated media produce gene mutation in the bacterium Staphylococcus aureus[1] and in the sexually reproducing lower plant Neurospora crassa[2]. The same mutation spectrum as with direct irradiation of the organisms was obtained. The mutagenic radiomimetic) action could be ascribed to certain hydroperoxides (See summaries [3, 4]). - The UV-effects will not be discussed further in this context.

3. Ionizing radiations.

3.1. Microorganisms.

3.1.1. Irradiated solid glucose and protein showed when tested on lysogenic bacteria no increase in lysogeny;[5] although this system is very sensitive to direct irradiation.[6] The experiments are perhaps not conclusive because too low doses were used (1-5 Mrad to solid compounds, cf. Appendix).

3.1.2. Most work with microorganisms was done with UV-irradiated media (See 2). A slight increase of mutation to streptomycin-independent in *E. coli* has been observed after treatment with a distillate from irradiated animal standard food.[9] The effect, which was observed to some degree also in the control with irradiated food, is open to criticism because of the low pH during treatment which may cause mutation by depurination.

3.1.3. It may be expected that especially microorganisms will show increased mutation rates when treated with X- and γ-irradiated media under conditions giving certain (hydro)peroxides.[10,11] (Cf. 4).

3.2. Higher plants.

Most of the available data in higher plants are cytological, describing chromosomal aberrations in root meristems induced by irradiated media and other systems (3.2.1.-3.2.9.). Certain plant materials present a good system for the detection of and screening for chromosomal aberrations. It should be noted, however, that species of the family *Leguminosae* are sensitive to an effect of anaerobiosis during germination (but not later divisions) producing chromosomal aberrations.[12,13] Cereals do not show such effects and data from these plants are therefore more reliable.[14] The same holds true for the "Allium test".[15]

Only few efforts have been made to measure mutation rates (3.2.10.).

3.2.1. Early experiments of Natarajan and Swaminathan[16] demonstrated that X-irradiated distilled water can induce chromosome breaks in wheat and onion root tips (See Appendix).

3.2.2. In another experiment it was observed that chromosome breaks were produced if the cereal embryos were grown in X-irradiated Whites' cultural medium (for composition, see Appendix). These experiments were repeated later with γ-rays by Natarajan (unpublished) and the above results were confirmed.

3.2.3. Irradiated thymine solution was found also to induce chromosome breaks in barley.[14] There was a low frequency of breaks induced by unirradiated thymine solution, but the frequency was significantly higher with irradiated thymine solution (se Appendix).

3.2.4. Moutschen and Matagne[19] studied the effect of solutions of irradiated glucose monohydrate (γ-ray doses from 0.05 to 74 Mrad) on barley and Allium roots. An elaborate analysis was made in this study. A 6 fold increase in the chromosome aberration frequency, over the control, was observed. The interesting observations made in this study was the lack of any dose dependent increase of breaks, the highest rate (9-10 %) being reached with the glucose irradiated with 0.5 to 1 Mrad. (See Appendix). The dose independence of these indirect effects has been observed in Drosophila[20] and mice also. (See Discussion).

3.2.5. Very few studies have been conducted in this respect with food or food components. Swaminathan et al.[21] studied the effect of irradiated potato mesh (20, 40, 80 kr) on barley root meristems by culturing barley embryos on the mesh. A large number of cells was analysed in this study which indicated the presence of a high frequency of laggards and micronuclei probably due to chromosome fragmentation. (See Appendix). When the embryos were grown (on the irradiated medium) in the presence of oxygen, the effect was considerably reduced. Chopra and Swaminathan[22] irradiated potato tubers with 20 kr and stored them at +4°C för 8 months, and then they were meshed, and used as medium for the culturing of barley embryos. An appropriate control was kept with unirradiated potatoes. The developing root meristems showed a considerable increase in cytological aberrations (mostly of micronuclei).

3.2.6. Chopra et al.[23] studied the effect of irradiated fruit juices, on the root meristems of barley and Allium. These was a distinct and significant increase in the chromosome aberration frequency of the treated material in comparison to the controls. (See Appendix). The characteristics of the type of breaks induced was the localization at the contromeric region (resembling certain effects of alkylating agents) and the absence of exchanges.

3.2.7. Unpublished results of De Stanchina have indicated that irradiated orange juice induces only mitotic inhibition, but no chromosomal aberrations. (See Appendix).

3.2.8. Moutschen et al. (unpublished) have also tested the possible radiomimetic activity of (a) straw berry powder irradiated with 0.5 to 5 Krads of 3 MeV β-rays (b) cooked potato powder - irradiated with 10 Krad (1 Mev) (c) mashed potato treated with 20-80 Krad of X-rays (d) raw potato treated with 16-70 Krad of γ-rays (^{60}Co) and (e) wheat flour + soya flour exposed to a total dose of 200 Krad of γ-rays. All results were negative.

3.2.9. Although of less connection with the problem of irradiated foods, a few other experiments showing indirect radiation effects may bereported. D'Amato and Moletti (personal communication) transplanted unirradiated embryos over presoaked endosperm irradiated with low doses (0.5 to 1 Krad) and found that 24 hours after transplantation 20 to 25 % aberrant rot tip cells at anaphases contained chromatid breaks and deletions frequently, and bridges very rarely.

Kuzin[27], in a series of studies,has demonstrated the presence of nucleotoxic substances in extracts of irradiated plant tissues, capable of reducing mitotic index as well as induce chromosome aberrations in other tissues. The activity has been attributed to the formation of polyphenols.

3.2.10. Gene mutation.

Very few experiments have been conducted to demonstrate the possible mutagenicity of irradiated compounds in higher plants. Ehrenberg[28] studied the effect of irradiated glucose and glycine (50×10^6 R of ^{60}Co γ-rays), on barley seeds. The chlorophyll mutations were scored in the second generation, while the irradiated substrate gave a mutation rate of 1 %, the unirradiated substrate gave 0.4 %; the effect being significant at 0.02 % level.[x)]

3.3. Drosophila.

This insect represents one of the most efficient genetic systems of the class of higher organisms. Several techniques for quantitative detection of mutation frequencies have been developed in this organism.

It may be mentioned that Kraybill suggested that Musca domestica, the house-fly, which affords similar possibilities, should be utilized in the evaluation of mutagenicity or carcinogenicity of degradative products arising from processing of food.[29]

In several experiments Drosophila has been fed with irradiated basic media (containing water, glucose, agar, yeast powder, and propionic acid) or with basic medium containing irradiated DNA.

x) When this experiment was repeated, no increase of mutation rate was obtained.

The results are contradictory, certain studies showing raised mutation rates, others no effect.

Chromosomal aberrations after feeding with irradiated medium were not studied in this material.

3.3.1. Swaminathan et al.[30] observed a 3 - 4 fold increase over the spontaneous rate of sex-linked recessive lethals after feeding with irradiated basic medium (150 Krad γ-radiation). The Muller - 5 technique was used following feeding of the wild type males from egg to adult stage. The males were then mated to females raised on unirradiated medium. The induced mutation is significant at $P < 0.01$ (cf. Appendix). Also visible mutants were registered

techniques.

3.3.2. Chopra[31] repeated the experiment of Swaminathan et al. (see 3.3.1), using X-chromosome recessive lethals. Certain differences were introduced, however, (a) a higher dose, (b) a changed composition of the medium (sucrose instead of glucose), (c) the media were not immediately used (transported from a far-off γ-source). No mutagenic effect was found. In view of the differences mentioned, in view of the smallness of the experiment, and in view of the fact that experimental details permitting the evaluation of clustering and, hence, a statistical treatment, the experiment does not contradict or invalidate the earlier[30] result. (See Appendix).

3.3.3. Moutschen and Moutschen-Dahmen[32] have fed both the OR-K and Muller-5 flies - with medium irradiated with 150 Krad, from 1 to 9 generations. The data gave a frequency of 18 cases out of 1366 (i.e. 1.32 %), in contrast to 3 cases in 1196 in the control (0.251 %). These data indicate a 5 fold increase in mutation rate. The statistical significance is high. (See Comments).

3.3.4. A detailed experiment, utilizing adequate statistical treatment, has been done by Rinehart and Ratty[20]. Flies were allowed to feed on an irradiated medium (irradiated with X-ray doses of 150, 500, and 3000 Krad). Two types of treatments were done: in one experiment, the medium was used immediately after irradiation and in another, the medium stored for 3 weeks after irradiation was used. There was a small but significant increase in the mutation frequency. (See Appendix). The same increase was obtained at all tested doses. The increase was found to be mainly from genial mutants (i.e.

increase of clusters of mutants) which was absent in the control, except in one case. In the series with immediate treatment there seems to be an increase in non-genial mutants, too (= post meiotic mutants). In the aged series there was a decrease in non-genial mutants, although it is indicated that genial mutation rates are still high.

3.3.5. It may be mentioned that Moutschen used the Muller-5 technique to determine the mutagenic effect of irradiated potatoes (8 Krad) and irradiated soya flour (200 Krad). No effect was found. Only 150-250 chromosomes were tested in each treatment. This size of the experiment is too small to detect mutation rates below around 5 %. The experiment is therefore not conclusive in any direction.

3.3.6. Irradiated DNA. Om Parkash[33,34] published recently results indicating a strongly increased mutation rate following admixture of irradiated DNA to the food. The DNA (fish sperm) was irradiated with 10^5 rad and added to the medium, in which eggs were laid. When testing sex-linked recessive lethals[33] (ClB technique), 5.7 per cent mutation was reported against 0 in the control (representative for standard average = 0.17 %). The experiment was not designed to detect clusters, and the increase is therefore not significant (see Appendix). In a test of II chromosome autosomal recessive lethals[34] a similar increase was found. In this case a test for clustering was done (personal communication) indicating that a high mutation rate was really obtained.

Two laboratories (Cf. Chopra[31] and Rinehart and Ratty[35]) have repeated Parkash's experiment on sex-linked recessive lethals with addition of irradiated DNA to the medium. In none of the experiments any mutagenic effect is shown, and the experiments are big enough to exclude, with a high significance, even a two-fold increase of mutation. Chopra also considers the possibility of yeast counteracting the mutagenic effect of irradiated DNA. His material on that point is not big, but seems to exclude the high mutagenicity found by Parkash, also in the absence of yeast.

Nobody has repeated Parkash's experiments with II chromosome lethals.

Although without direct inference to effects of feeding irradiated food, it should also be recalled that Fahmy and Fahmy[36] failed to find lethals following injection of irradiated Drosophila

DNA, in the vicinity of the gonads of the flies. (In these experiments it was found, however, that DNA degraded by ultrasonics or irradiation and then injected is able to induce "minutes", i.e. small deletions which may be of genetical significance.

We may conclude from these experiments that irradiated DNA does not induce mutation generally (and that it certainly does not require special attention in the evaluation of the wholesomeness of irradiated food). The results of Parkash are scientifically very interesting, however, and a comparison of the DNA used in his tests with other products are recommended.

3.4. Mammals.

3.4.1. Data from multigeneration feeding tests. - The large-scale animal feeding tests performed in order to establish the wholesomeness of irradiated foods have not been designed in a way permitting an estimation of possible genetical hazards. It is true that no significant (or clearly accidental) effects have been found on the breeding of the animals in multigeneration tests (see e.g. reports on feeding tests with irradiated pork,[37] irradiated potatoes[38] and irradiated wheat and wheat products[39]). But the litter size may be affected by trivial dietal factors influencing e.g. the number of corpora lutea etc., and it is further a very insensitive measure of lethal mutation rate (cf. Lüning's[40] studies of dominant lethal mutations in irradiated mice). Therefore, the litter size and the breeding of the animals cannot be used for the estimation of mutation in mammals.

3.4.2. No studies of genetical effects in mammals are at present published, but a few cytological and also genetical experiments in mice of a more or less preliminary character have been run chiefly by J. and H. Moutschen-Dahmen, partly in collaboration with L. Bugyaki and A. Lafontaine.

3.4.3. Chromosomal aberrations in mice.

All experiments to be reported utilize spermatogonial divisions in mice as object of study. Studies of dividing lymphocytes in rats, under lymphoponic conditions (see 3.4.5.) produced by irradiated

conclusive these studies require a further development of the phytohemagglutinin technique for rat lymphocytes.

3.4.3.1. J. and M. Koutschen-Dahmen (unpublished) studied preliminarily in 5 exposed and 5 control mice the aberrations produced by irradiated "Hosby" food (for composition, see ref.[41]). The former exhibited an increased rate significant at P ≈ 0.05.

3.4.3.2. The same investigators assayed the influence of irradiated glucose, dissolved and given to the mice in the drinking water during 48 hours (somewhat arbitrarily chosen time, found to the suitable in corresponding tests with alkylating chemicals[42]). The glucose was irradiated as the monohydrate, i.e. the modification the radiation chemical products of which are most efficiantly killing bacteria[7]. The experiments were run as parallels to the botanical studies published (see 3.2.4.).

In the first series comprising irradiation with 5 Mrad and preparation of a 2 % solution the spermatogonial anaphase and the two spermatocyte anaphases were studied. In the five exposed animals 28 aberrant among 871 investigated cells were found, in comparison to 7 cells out of 800 in the five control animals given unirradiated glucose. The difference is strongly significant if we consider the aberrant cells independent (i.e., if no grouping to certain sensitive animals occurs).

In the second series/glucose irradiated with 74 Mrad was given under similar conditions. The summarized results (cf. Appendix) shows 34 aberrant out of 1235 studied cells in the five test animals compared to 3 aberrations in 1016 cells of the five control animals. Even in this case, and under conditions of independence of aberrant cells a high significance is obtained for the difference.

It may further be stated that in each of the divisions studied, the same increase of aberration rate was obtained in the two series, although the radiation dose of the latter was 15 times higher.

3.4.3.3. In cooperation with Bugyaki and Lafontaine a bigger experiment with 30 exposed and 30 control mice was run with irradiated food. 50% of a standard animal diet similar to the Hosby food was irradiated. The males were treated for 4 months from weaning. The results are summarized in Table 1, which also gives the aberration types observed. It appears that the frequencies of cells with aberrations are around 3 times higher in the animals exposed to irradiated food compared to the controls, and this increase is highly significant.

3.4.3.3. Table 1. General proportions of disturbed cells.

I. Animals fed irradiated food.

Metaphase I		Anaphase I		Anaphase II		Gonial anaphase	
Number of analysed cells	Number of disturbed cells	Number of analysed cells	Number of disturbed cells	Number of analysed cells	Number of disturbed cells	Number of analysed cells	Number of disturbed cells
1028	8	1212	33	1743	20	1962	33
II. Control animals.							
1028	1	1005	8	1273	6	1838	11

3.4.3.3. Table 2. Proportion of aberration types.

I. Animals fed irradiated food.x)

Metaphase I		Anaphase I		Anaphase II		Gonial anaphase	
Translo-cations	Other dis-turbances	Bridges	Fragments	Bridges	Fragments	Bridges	Fragments
7	1	25	18	9	13	16	20
II. Control animals.							
0	1	2	7	3	4	7	11

x) Numbers of analysed cell the same as in Table 1.

In order to evaluate the significance the statistics most disadvantageous to the experiment may be used assuming a maximum grouping of the aberrations to certain "abnormal" animals, restricting the analysis to animals with and without aberrant cells (cf. Table 3). Nearly all treated animals exhibit aberrations, in contrast to about half of the control animals. P = 0.0017 for the hypothesis that the frequencies of animals with aberrations are similar in the two groups.

3.4.3.4. Table 3.

Observation	Experiment with complete irradiated diet (3.4.3.3.)		Experiment with irradiated glucose (2nd series, 74 Krad, 3.4.3.2.)	
	Exposed animals	Control animals	Exposed animals	Control animals
Animals with no aberrations	3	14	0	4
Animals with aberrations	27	16	5	1
Of these, animals with translocations	5	0	2	0

The frequencies of animals exhibiting translocations (observed at metaphase) may be treated in the same way. In the treated series the 7 translocations found are distributed among 5 animals, compared to 0 such animals in the control series. P for equal distribution is about 0.03. To this series may be added the second glucose series, with 2 animals against 0 with translocations (cf. Appendix 3.4.3.2. Table 2). Testing the hypothesis that 7 out of 35 represent the same frequency as 0 out of 35, we find P = 0.006.

3.4.4. Mutation.

3.4.4.1. K.G. Lüning (unpublished) performed one experiment with mice where males fed irradiated food of the type demonstrated to produce a lymphopenia in rats.[41] Since the lymphopenia corresponded to that induced by about 30 rad total body γ-irradiation, the experiment was designed to detect the frequency of dominant lethal mutations induced by this dose in spermatids - sperms. The result was negative, the expected increase of the frequency of dead implantations not being found. (See Appendix).

3.4.4.2. J. and K. Moutschen-Dahmen have run a few preliminary experiments with different age of animals and different feeding periods. In large, the results agree with those of Lüning in the fact that, when males were fed the irradiated diet, no significant increase of decidua (per corpora lutea or per implantation) was found.

It was strongly indicated, however, that when both sexes were fed the irradiated food (the feeding of females with irradiated food was interrupted before gestation) the frequency of dead implants was increased by a factor 2-3. ($P \approx 0.01$ for equality of test series and control). (See Appendix).

We regret that this important work had to be discontinued for financial reasons at a moment certain interesting results started to appear, and a continuation of this type of work is strongly recommended.

In order to avoid misinterpretation we want to stress the extremely preliminary nature of Moutschen's data. Many factors of a more or less trivial nature may influence the shedding of eggs and their fertilization and early development, with a possibility to disturb the determination of dominant lethals. The number of corpora lutea per female seems thus to be affected by minor factors in the food, such as heating to 80° during the preparation of an

otherwise unchanged standard diet;[40] (hence, no conclusions as to absence or presence of lethal mutations should be drawn from an unchanged or decreased average litter size). It should also be mentioned that a control series (of a ^{90}Sr experiment) of mouse males receiving NaCl injections exhibited high frequencies of "dominant lethals" when mated within 3 weeks after injection; the reasons for this behavior is being investigated.[43]

It is extremely important to verify Moutschen's observation of increased number of decidua, with well comparable[x)] controls and series where males, or females, or both sexes are fed irradiated diets during different periods of the life span. The intra-uterine death observed after feeding both sexes may indicate a raised mutation rate, but it may also indicate a non-tolerable somatic damage during foetal development.

3.4.5. Although a leukopenia (lymphopenia) may be produced by many disturbances (see e.g. lists presented by Wintrobe[44], p. 1098) other than radiations, radiominetic chemicals, and antimetabolites affecting the genetic material, this effect, which has been clearly established to be produced in the rat by irradiated food,[41] glucose,[45] etc., may be mentioned in this context. The lymphopenia is easily demonstrated in a few animals, and the experimental conditions, under which this effect demonstrated that <u>something</u> (with a possible relationship to genetical damage) had happened to the animals, were used in the genetical studies of the Moutschen-Dahmen team. Due to the great difficulties and high costs involved in the testing of mutagenicity in mammals (and, especially, if the mutation rates are low) we find it recommendable that the procedure, in certain experiments at least, should consist in the two steps mentioned

(a) establishment of optimum conditions for development of any "radiominetic" effect;

(b) test of mutagenicity and chromosomal aberrations under this set of conditions.

If the test (b) is positive, it must of course be established

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x) with respect to age, location in the room, earlier litters, etc.

4. Discussion.

4.1. Established experimental facts.

In certain well-investigated series it has been clearly demonstrated that irradiated food (media, compounds) are able to induce gene mutation (as established by genetical tests) and/or chromosomal aberrations in organisms belonging to different classes: microorganisms, plants, insects, mammals.

The effects are mostly obtained in the range of doses, 10^5 - $5 \cdot 10^6$ rad, intended for radiopasteurisation and radiosterilisation.

In all investigated materials the frequencies of genetical changes are low - in many cases we are dealing with an increase by a factor 2-3.

In certain cases no effect was found. Such negative results were partly obtained under experimental conditions very similar to these giving positive results. It is most probable that the negative results do not invalidate the positive ones and v.v. We should rather draw the conclusion that still unknown variables in the test organism or in the food irradiated may affect the reaction pattern with the consequence that genetical damage is either induced or not induced. This increases the difficulties of analysis, but may be guiding in efforts to unravel the action mechanisms.

In a few cases the influence of radiation dose was investigated:

Drosophila, medium	0.15 - 3 Krad
Mouse, crystalline glucose	5 and 74 Krad
Hordeum roots, glucose	0.05 - 74 Krad

In each case all the investigated doses give approximately the same mutation (Drosophila) or aberration (mouse, Hordeum) rate, respectively.

4.2. Action mechanisms.

The nature of the radiations used (mostly therapy X-rays or 60

effects observed will therefore be due to radiation chemical changes in the irradiated food.

The absence of dose dependence in any case where the dose dependence was investigated, indicates that we are not dealing with principles with a reactivity vis-a-vis the gene material like

most radiomimetic alkylating chemicals. It is known that certain radiation chemical products, such as H_2O_2, may reach a steady state concentration maintained in a broad range of concentrations, but the possibility that reactive hydroperoxides are involved is less likely, partly in view of the fact that such compounds are not formed when irradiated crystalline glucose is dissolved in water (G. Löfroth and L. Ehrenberg, unpublished).

The absence of dose dependence (and partly the fact that no genetical change was found in certain cases) indicates that the genetical effects we observe are induced in an all-or-none response to a trigger mechanism, which is released when certain active compounds exceed a threshold dose. The degree of response will then be predetermined by physiological properties of the cell population exposed to the active compounds.

It is true that also a lack of an essential vitamin etc. may produce a physiological-biochemical change of the kind required for a changed mutation rate. In the animal experiments described such an explanation is less plausible, however, because only a fraction of the total, supervitaminized diet was irradiated. It has further been shown that other biological effects can be released by compounds which can be extracted from irradiated glucose solution[8] and irradiated animal diet.[9]

The dose-independent all-or-none character of the genetical effects resembles the properties of the rat lymphopenia induced.[41,9] In this case a possible "trigger" is indicated:
The effect may partly at least be restored by folic acid given in the drinking water.

A direct or indirect anti-folic acid effect of irradiated foods would disturb the synthesis of thymidylate, i.e. a DNA precursor, in a way which might give chromosomal aberrations (as shown by Taylor for aminopterin[46]) or gene mutation (as shown by Heslot[47] to be induced in yeast by aminopterin and metathrexate). It should be observed that other investigators have found folic acid antagonists to be inactive in this respect.[48,49]

As another guide-line for further research it should be recalled that vegetable oils are able to produce chromosomal aberrations and gene mutation in higher plants.[50] This effect is exaggerated in methyl linolate[51], i.e. an unsaturated compound separated from the normal antioxidants. Since ionizing radiations

tend to destroy natural antioxidants, the possible consequences of this should be observed. (Compare with the fact that irradiation of oils- at high doses - has been demonstrated to induce a considerable toxicity to test animals[52]).

4.3. Possible consequences to human consumption of irradiated food.

The data presented in this report do not justify us to judge the genetical consequences to man of a general use of radiation preserved food. (The same concerns, of course, animals fed irradiated fodder.) At the present state of affairs genetical effects can be neither proved nor excluded.

In several instants a 2-3 fold increase in mutation rate in a broad sense, was observed to be induced, independently of the dose, in Drosophila and mice, and such an increase of mutation rate in large human populations would not be tolerated according to current principles for radiological protection. ICRP sets the "maximum permissible genetic dose",[53] given to members of the public, to 5 rem, a dose expected to increase the mutation rate by 5-50 per cent.[54]

It should be noted, however, that no food of a type intended for human consumption was tested in any of the animals (although it has been indicated that related effects in plants are produced by irradiated fruit juices, potatoes, etc.). We do not think that such tests are yet possible, e.g. with mice. The development of reliable techniques is dependent on further work with those food types, i.e. standard animal diets, which have already given certain genetical effects. These experiments must be repeated, and a series of essential variables must be evaluated.

We should further note that only the Drosophila experiments prove that gene mutation sensu strictu is induced by irradiated media. The chromosomal aberrations found in plants and mice are mostly of types eliminated before they can reach the following generation; in plants, certain aberrations have been demonstrated to be transferred, however, and this will probably, when investigated, also be the case in mammals.

It may also be said about the dominant lethal mutations, measured as intrauterine death, that if the effect is restricted to such early foetal death, the harm will be limited.

The genetical changes studied represent such effects which are most easily studied, and they do not coincide directly with

such more harmful effects the frequencies of which we want to estimate. It may be said, however, that most agents inducing any of the effects studied give rise to all other genetical effects including the most dangerous ones, and we have to accept this as a working hypothesis also for the irradiated foods until we have proved the spectrum of effects to be undangerous. If, as suggested, antimetabolic action mechanisms are involved, a very skew spectrum of effects would not be unexpected, however; (antimetabolites like nebularine and 8-ethoxycaffeine are known to induce restrictedly either gene mutation or chromosomal aberrations, respectively.[55])

The high frequencies of intrauterine death found in one experiment to follow feeding irradiated food to females, may as well be a measure of somatic damage to the foetus. If this damage includes types where the children are born alive a non-genetical but nevertheless very harmful effect may be indicated. From the American multigeneration tests with irradiated foods no increase of living, abnormal offspring was reported; this would indicate that such somatic damage is rare. (When such somatic effects are estimated it is important to chose the right test animals, however, as demonstrated by recent experience from tests with pharmaceuticals.)

5. Conclusions and proposals.

From the experiments herein reported it is impossible to arrive at any definite conclusion as to the presence or absence of genetic effects if irradiated food were used for human consumption or for animal feeding. Hitherto available data indicate, however, that increased rates of mutation and chromosomal aberrations will probably be induced in certain cases. Although experiments indicate that the genetical effect, in cases where it is induced, is relatively small compared to the effect of direct exposure of mammals to radiation, the same experiments indicate that the possible effect will not be negligible. The intrauterine death described may indicate lethal mutations or somatic damage to the foetus. Although non-genetical in principle the latter

For this reason new experiments with careful layout and strict coordination are needed. Especially when different laboratories obtain contradictory results, exchange of food samples and test organisms is recommended.

5.1. Since cytological aberrations, as chromosome breaks and translocations, are events implying a mutagenic effect, because of the possible formation of clones of mutated cells in the somatic tissues or in the gonads, the quick tests on chromosomes of higher plants are still required. Root meristems of cereal seedlings or onion bulbs can be employed. Cytological analyses should be performed in a way permitting the determination of not only the possible damage (absence of mitoses, chromosome breakage and rejoining) but also of the nuclear stage in which the possible action took place.

5.2. Relatively quick proofs of genetic effects can be obtained using microorganisms and lower animals, as Drosophila. In any case, the investigations must be carried out with different species. The analyses proposed under the items 5.1. and 5.2. seem useful and necessary; they complement each other. Basic differences, in fact, exist between the biological systems involved.

5.3. Experiments with "in vitro" tissue cultures should be carried out along with the development of this technique.

5.4. Undoubtedly, the definitive genetic approach, the results of which can be rather correctly extrapolated to man, has to be performed on different species of small mammals. Because of the complexity, cost and duration of such investigations, it might be convenient first to develop the techniques and clarify the influence of essential variables in well-planned experiments with those irradiated standard diets which induce chromosomal aberrations in mouse spermatogonia and possibly also intrauterine death in the same material. Tests with other foods should be done after they have been found effective in microorganisms, higher plants, or Drosophila, or under conditions giving a lymphopenia in rats.

5.5. If feeding tests with human volunteers (of the type already conducted in the US studies) are again started, indications of genetical changes in the test persons may be obtained from a study of chromosomal aberrations in phytohemagglutinin stimulated lymphocytes. For this purpose the background values (and X-ray pre-history, etc.) of the test persons should be determined in advance. The result of such a test should be judged in view of the ratio, which should be determined, of lymphocyte aberrations to gene mutation in animals.

13. Rieger, R., and Michaelis, A. (1958) Cytologische und Stoffwechselphysiologische Untersuchungen am aktiven Meristom der Wurzelspitze von Vicia faba L. I. Der Einfluss der Unterwasser-Quellung der Samen auf die chromosomale Aberrations-rate. - Chromosoma 9, 238-257.

14. Natarajan, A.T. (1960) Stored energy and indirect biological effects of radiations. - Risö Report 16, 39-40.

15. Levan, A. (1951) Chemically induced chromosome reactions in Allium cepa och Vicia faba. - Cold Spring Harbour Symp. Quant. Biol. 16, 233-243.

16. Natarajan, A.T., and Swaminathan, M.S. (1958) Indirect effects of radiations and chromosome breaks. - Ind. J. Genet. 18, 220-223.

17. Scholes, G., Weiss, T., and Wheeler, C.M. (1956) Formation of hydroperoxides from nucleic acids by irradiation with X-rays in aqueous systems. - Nature 178, 157.

18. Chevallier, M.R., and Luzatti, D. (1960) Action mutagène spécifique de trois peroxydes organiques sur les mutations reversis de deux loci de E. coli T-9-13. - Compt. rend. acad. sci. 250, 1572-1574.

19. Moutschen, J., and Matagne, R. (1965) Cytological effects of irradiated glucose. - Rad. Botany 5, 23-28.

20. Rinehart, R.M., and Ratty, F.J. (1965) Mutation in Drosophila melanogaster cultured on irradiated food. - Genetics (in press)

21. Swaminathan, M.S., Chopra, V.L., and Bhaskaran, S. (1962) Cytological aberrations observed in barley embryos cultured in irradiated potato mash. - Rad. Research 16, 182-188.

22. Chopra, V.L., and Swaminathan, M.S. (1963) Sprout inhibition and radiomimetic properties in irradiated potatoes. - Die Naturwissenschaften 50, 374-375.

23. Chopra, V.L., Natarajan, A.T., and Swaminathan, M.S. (1963) Cytological effects observed in plant material grown on irradiated fruit juices. - Rad. Botany 3, 1-6.

24. Moutschen, J., and Moutschen-Dahmen, M. (1958) L'action du Myleran (Di-méthane-sulfonyloxy-butane) sur les chromosomes chez Hordeum sativum et chez Vicia faba. - Hereditas 44, 415-446.

25. Swaminathan, M.S., Chopra, V.L., and Bhaskaran, S. (1962) Chromosome aberrations and the frequency and spectrum of mutations induced by ethyl methanesulfonate in barley and wheat. - Ind. J. Genet. 22, 192-207.

26. Natarajan, A.T., and Upadhya, M.D. (1964) Localized chromosome breakade induced by ethyl methanesulfonate and hydroxylamine in Vicia faba. - Chromosoma 15, 156-169.

27. Kuzin, A.M. (1962) On the role of the disturbance of metabolic processes in the radiation damage of the cell. - Int. J. Rad. Biol. 6, 211-220.

28. Ehrenberg, L. (1960) Chemical mutagenesis. Biochemical and chemical points of view of mechanisms of action. - Abh. Deut. Akad. Wiss. Berlin, Kl. Med. Jahr. 1960 Nr 1, 124-136.

29. Kraybill, H.F. (1962) General methods and problems in assesment of potential toxicity and carcinogenicity in Radiation and thermal processed foods and food additives. - Report of the FAO/WHO/IAEA Meeting on the Wholesomeness of Irradiated Foods. Brussels, 112-122.

30. Swaminathan, M.S., Nirula, S., Natarajan, A.T., and Sharma, R.P. (1963) Mutations incidence in Drosophila melanogaster reared on irradiated medium. - Science 141, 637-638.

31. Chopra, V.L. (1965) Tests on Drosophila for the production of mutations by irradiated medium or irradiated DNA. - Nature (in press).

32. Moutschen, J., and Moutschen-Dahmen, M. (unpublished).

33. Parkash, Om (1965) Induction of sex linked recessive lethals and visible mutations by feeding X-irradiated DNA to Drosophila melanogaster. - Nature 205, 312-313.

34. Parkash, Om (1965) On the radiominetic effect of irradiated desoxy ribonecleic acid (DNA_m) on Drosophila melanogaster. - Die Naturwissenschaften 6, 142.

35. Rinehart, R.R., and Ratty, F.J. - Personal Communication.

36. Fahmy, O.G., and Fahmy, M.J. (1965) Genetic properties of exogenous deoxy ribonucleic acid at various levels of degradation in Drosophila melanogaster. - Nature 207, 507-510.

37. Bubl, E.C., and Butts, J.S. (1960) The growth, breeding and longevity of rats fed irradiated or non irradiated pork. - J. Nutrition 70, 211-218.

38. Okouneva, L.A. (1958) Valeur hygiénique de la pomme la pomme de terre irradiée par le cobalt radioctif pour arrêter la germination. - Vopr. Pitania, S.S.S.R. 17, 49-53.

39. Brownell, L.E., Horne, T. and Kretlow, W.J. (1962) Petition for the use of gamma radiation to process wheat and wheat products for the control of insect infestation. (miniografed manuscript)

40. Lüning, K.G. (1964) Studies of irradiated mouse populations III. Accumulation of recessive lethals. - Mutation Research 1, 86-98.

41. Ehrenberg, L., Löfroth, G., and Ehrenberg, A. (1965) Biological effects of irradiated food. I. Effect on lymphocyte numbers in the peripheral blood of the rat. - Arkiv. Zool. (in press).
See also
Ehrenberg, L. (1962) The concept of stored energy (with special regard to radiomimetic effects produced by irradiated foods).- Report of the FAO/WHO/IAEA Meeting on the Wholesomeness of Irradiated Foods. Brussels, 166-178.

42. Moutschen, J. (1961) Differential sensitivity of mouse spermatogenesis to alkylating agents. - Genetics 46, 291-299.

43. Lüning, K.G. et al. (unpublished).

44. Wintrobe, M.M. (1961) Clinical hematology. - Henry Kimpton, (London).

45. Ehrenberg, L., and von Ehrenstein, G. (1960) How should a possible carcinogenic action of irradiated food be measured? - Risö Report 16, 41-43.

46. Taylor, J.H. (1963) DNA synthesis in relation to chromosome reproduction and the reunion of breaks. - Symp. Macromolecular Aspects of the Cell Cycle: J. Cell. Comp. Physiol. 62, 73-86.

47. Heslot, H. (1960) Note sur l'activité mutagène de certains antifoliques. - Abhandlungen der Deutschen Akademie der Wissenschaften zu Berlin. Jahrgang 1960, 1, 8.

48. Moutschen, J. (unpublished).

49. 1.Truhaut, R., and Deysson, G. (1963) Caractéristiques de l'activité antimitotique de l'aminopterine sur le test Allium. - C.R. Soc. Biol. (Paris) 157, 1963-1965.
2.Deysson, G., and Truhaut, R. (1963) Influence de l'acide folique et de l'acide folinique sur la cytotoxicité de l'aminopterine: études sur le test Allium. - C.R. Soc. Biol. (Paris) 157, 2179-2182.

50. Swaminathan, M.S., and Natarajan, A.T. (1959) Cytological and genetic changes induced by vegetable oils in Triticum. - J. Heredity 50, 170-187.

51. Natarajan, A.T., and Ehrenberg, L. (unpublished).

52. Lang, K. (1962) Studies on the wholesomeness of irradiated fats.- Report of the FAO/WHO/IAEA Meeting on the Wholesomeness of Irradiated Foods. Brussels, 148-156.

53. I.C.R.P. Publication 6. - Pergamon Press, 1964.

54. Report of the United Nations Scientific Committee on the Effects of Atomic Radiation (1958) - General Assembly, Official Records, 13th session, Suppl. No. 17 (A/3838).

55. Ehrenberg, L., Gustafsson, Å., and Lundqvist, U. (1956) Chemically induced mutation and sterility in barley. - Acta Chem. Scand. 10, 492-494.

56. Russel, W.L. (1954) Genetic effects of radiation in Mammals. - Radiation Biology (Ed. A. Hollander, Mc Graw Hill) 1, 825-860.

57. Eriksson, G. (1963) Induction of waxy mutants in maize by acute and chronic gamma irradiation. - Hereditas 50, 161-178.

58. Ehrenberg, L., and Eriksson, G. (1964) Mutation in the waxy character in barley pollen grains following ^{90}Sr-incorporation at low activities. - Mutation Research, 1, 139-145.