mutation rate with increasing dosage of fast neutrons appeared to be linear for sex-linked lethals, both with and without gross chromosomal aberrations; but the rate may not have been linear in the case of the autosomal visibles.

* This work was a part of U. S. Atomic Energy Commission project 23.4 and was supported in part by funds from U. S. Atomic Energy Contract No. AT (30-1)-930 and in part by a grant-in-aid from the Rockefeller Foundation.

† President address, Department of Biology, Harvard University, Cambridge, Mass.

[1] Alexander, Mary L., *Rec. Genetics Soc. Am.*, **21**, 7 (1952).

[2] Young, W. J., Yost, H. T., Jr., Ives, P. T., and Levine, R. P., these PROCEEDINGS, **39**, 488–495 (1953).

[3] Ives, P. T., *Evolution*, **4**, 236–252 (1950).

[4] Hinton, T., Ives, P. T., and Evans, Alice T., *Ibid.*, **6**, 19–28 (1952).

[5] Dempster, E. R., these PROCEEDINGS, **27**, 249–250 (1941).

[6] Giles, N. H., Jr., *Genetics*, **28**, 398–418 (1943).

[7] Lea, D. E., *Actions of Radiations on Living Cells*, Macmillan Co., New York, 1947, 402 pp.

*PROTECTIVE PROPERTIES OF CYSTEINE, SODIUM HYPOSULFITE, AND SODIUM CYANIDE AGAINST RADIATION INDUCED CHROMOSOME ABERRATIONS**

BY KNUT MIKAELSEN†

BIOLOGY DEPARTMENT, BROOKHAVEN NATIONAL LABORATORY, UPTON, NEW YORK‡

Communicated by Karl Sax, December 17, 1953

Introduction.—Several investigators have shown the importance of the presence of oxygen to the process of chromosome breakage during exposures to x-rays. By lowering the oxygen tension below 2 per cent or by exposing tissue to x-rays in the presence of other gases such as nitrogen and helium, a considerable decrease in the frequencies of chromosome aberrations was obtained. This was shown by Thoday and Read[1] in the root tips of *Vicia faba*, by Hayden and Smith[2] in barley seeds, and by Giles and Riley[3, 4] in microspores of *Tradescantia paludosa*. By removal of most of the oxygen in the tissue the frequency of x-ray induced aberrations was reduced to nearly one-third of that obtained when the tissue was exposed in air or in higher oxygen tensions. Apparently, it is also possible to obtain a similar reduction in aberration frequencies with chemicals. Mikaelsen[5] has shown that reduced glutathione added to the nutrient solution of *Tradescantia paludosa* reduced the frequency of chromosome fragments by about 50 per cent after a 48-hour exposure to chronic γ-radiation at a dose rate of 25 r/day. The results obtained with glutathione suggested testing of other chemicals for protection against radiation induced chromosome aberrations. In the present paper the effect of cysteine, sodium hyposulfite, and sodium cyanide are reported.

Material and Methods.—Cuttings of *Tradescantia paludosa* (clone B2-2) were used as experimental material. Root development was initiated by placing the cuttings in tap water with continual aeration at ordinary greenhouse temperature (65–70°F.).[5] When the cuttings showed good development of primary roots, they were transferred to specially designed lucite vessels which contained Hoagland and Snyder's nutrient solution.[6] The vessels were 30 × 2 × 12 cm. in size. The side of the vessel facing the source was 3 mm. thick.

After 24 hours in the nutrient solution the cuttings were transferred to fresh nutrient solutions containing the various concentrations of chemical agents to be tested. After one hour, the plants, still in the solution of the chemical compound, were exposed to continuous γ-irradiation from a CO^{60} source for 48 hours. In the experiments with cysteine-HCl and sodium hyposulfite§ a 3 curie CO^{60} source was used. The source was located in a cylindrical, stainless steel container with a front wall thickness of 0.8mm. and 1 mm. thick side walls. This source holder was placed

TABLE 1

THE EFFECT OF DIFFERENT CONCENTRATIONS OF CYSTEINE, SODIUM HYPOSULFITE ($Na_2S_2O_4$) AND SODIUM CYANIDE (NaCN) ON THE FREQUENCIES OF CHROMOSOME FRAGMENTS AND BRIDGES DURING EXPOSURE TO γ-IRRADIATION

CONCENTRATION USED	NO. OF CELLS SCORED	NO. OF FRAGMENTS	FRAGMENTS PER 100 Cells	PROTECTION, %	NO. OF BRIDGES	NO. OF BRIDGES PER 100 CELLS
		(*a*) Cysteine-HCl (low pH)				
None	614	212	34.5	0	10	1.63
1×10^{-5} *M*	403	118	29.3	15	7	1.74
1×10^{-4} *M*	606	117	19.3	44	12	1.98
1×10^{-3} *M*	595	97	16.3	53	10	1.68
		(*b*) Cysteine-HCl (low pH)				
None	668	113	16.9	0	14	2.09
3×10^{-4} *M*	548	37	6.8	60	12	2.19
3×10^{-3} *M*	Toxic	...	...	...	...	...
1×10^{-2} *M*	Toxic	...	...	...	...	...
		(*c*) Cysteine-HCl (high pH)				
None	313	76	24.3	0	12	3.83
5×10^{-4} *M*	472	90	19.1	21	8	1.69
5×10^{-3} *M*	498	85	17.1	30	13	2.61
1×10^{-2} *M*	585	92	15.7	35	17	2.91
		(*d*) $Na_2S_2O_4$				
None	141	33	23.4	0	5	3.55
1×10^{-5} *M*	149	19	12.8	46	5	3.36
1×10^{-4} *M*	211	29	13.7	41	5	2.29
1×10^{-3} *M*	Toxic	...	...	...	...	...
		(*e*) NaCN				
None	569	105	18.5	0	19	3.34
1×10^{-4} *M*	696	115	16.5	11	20	2.87
5×10^{-4} *M*	103	17	16.5	11	5	4.85
		(*f*) NaCN				
None	239	54	22.6	0	19	3.77
1×10^{-3} *M*	178	30	16.9	25	4	2.25

in the center of a spherical lead pig with a cone-shaped opening of about 70° for the beam. In the experiments with sodium cyanide an 8.5 curie CO^{60} source was used. The source was located in a similar source holder and was placed in a cylindrical aluminum pipe with a wall 1 mm. thick. The pipe was located near the corner of an L-shaped lead and concrete shield, which gave an irradiation field of about 90°. Dosages, as measured by the film badge method,[7] indicated that the plants in all the experiments received approximately 25 r/day and hence a total dose of 50 r.

Immediately following the 48-hour exposure period the root tips were fixed in alcohol-glacial acetic acid (3:1). Slides were prepared using the propionic- and aceto-carmine squash technique.

In the experiments reported below the following chemicals were tested: cysteine-HCl, sodium hyposulfite ($Na_2S_2O_4$), a strong reducing agent, and sodium cyanide (NaCN).

The number of both acentric fragments and bridges per 100 cells in anaphase of meristematic root tip cells was determined. However, conclusions regarding the effects of chemical treatments were based upon the fragment data only, since bridges occurred in such low frequency that it was difficult to evaluate the data.

The Influence of Various Chemicals on the Frequencies of Chromosome Fragmentation.—The frequencies of acentric fragments in the untreated control series exposed to chronic γ-irradiation were about the same in 4 of 5 experiments and varied from 16.9 to 24.3 per cent (table 1 (*b*), (*c*), (*d*), (*e*), (*f*)). However, the control series in the remaining experiment had a much higher number of fragments (34.5 per cent, table 1 (*a*)). The reason for this high value is not clear.

(*a*) *Cysteine Hydrochloride (Low pH)*: Cysteine-HCl was applied in two different experiments (table 1 (*a*) and (*b*)). In the first experiment (table 1 (*a*)) the following concentrations of cysteine-HCl were used: 10^{-5} *M*, 10^{-4} *M*, and 10^{-3} *M*. The presence of cysteine-HCl lowered the pH of the nutrient solution in which the plants were grown, particularly with the two strongest concentrations. The pH of the one-day old nutrient solution in which the plants were kept was about 5 just before the irradiation exposure started. After 48 hours the pH of the solution increased to about 6.6. In 10^{-5} *M* cysteine-HCl the pH was slightly lower, 4.5 and 6.2, respectively, before and after the experiments. At the beginning of the experiment the pH was 2.5 and 3, respectively, for the stronger concentrations, i.e., 10^{-3} *M* and 10^{-4} *M*. By the end of the experiment the pH values had increased to 4.2 and 4.6, respectively, for the same concentrations. Thus, the plants were grown and exposed to cysteine under quite different pH values. Judging by mitotic activity, the different pH values did not seriously disturb the roots since equal numbers of dividing cells were present in all cases.

In the first experiment the control series gave a fragment frequency of 34.5 per 100 anaphase cells after chronic γ-irradiation. In the presence of cysteine at a concentration of 10^{-5} *M* the number of fragments decreased slightly to 29.3 per 100 cells. Further decrease in fragment frequency was obtained with increasing cysteine concentrations. Treatments with 10^{-4} *M* and 10^{-3} *M* solutions gave, respectively, 19.3 and 16.3 fragments per 100 cells. The latter was a decrease of 53 per cent over that obtained in the control, which was the maximum effect in that experiment.

In another experiment the concentrations 3×10^{-4} *M*, 3×10^{-3} *M*, and 10^{-2} *M* were tested. Both 3×10^{-3} *M* and 10^{-2} *M* proved to be toxic to the roots. The roots became soft and the cells were damaged and unsuitable for studies. In the 3×10^{-4} *M* cysteine-HCl solution, however, the number of fragments per 100 cells was reduced from 16.9 which was obtained in the control to 6.8. This is a reduction in fragment frequencies of 60 per cent, which is the maximum effect obtained with cysteine. In figure 1 the results from these two experiments are plotted in curve *A* on a semilogarithmic scale. (*A* linear relation between protective ability and concentration is suggested.)

(*b*) *Cysteine-HCl in Partly Neutralized Medium of pH ~ 6:* At the beginning of another experiment where cysteine-HCl was used, each cysteine solution was adjusted to a pH of approximately 6 by the addition of potassium hydroxide. This was also the pH of the nutrient solution. Cysteine hydrochloride was used in concentrations of 5×10^{-4}, 5×10^{-3} and 10^{-2} *M*. Thus, in this experiment all the mixtures, those containing cysteine and the control, were exposed to irradiation at the same pH value. The last two concentrations were stronger than those previously employed. In this experiment (table 1 (*c*)) the fragment frequency fell from 24.3 per 100 cells in the control to 15.7 with 10^{-2} *M* cysteine, which was the

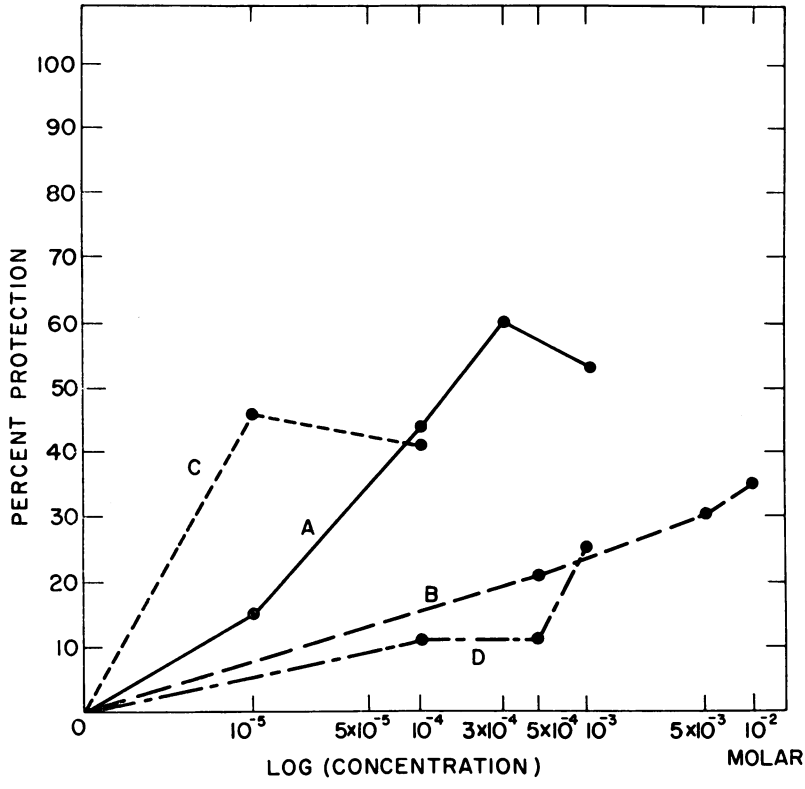

FIGURE 1

Relationship between concentration of chemicals used and percentage of protection. *A*, Cysteine, low pH. *B*, Cysteine, high pH. *C*, Sodium hyposulfite. *D*, Sodium cyanide.

strongest concentration used in the experiment. The intermediate concentrations, 5×10^{-4} and 5×10^{-3}, yielded fragment frequencies of 19.1 and 17.1, respectively, per 100 cells. The relation between per cent protection and concentration is plotted on a semilogarithmic scale (Fig. 1 (*B*)). There is an almost straight line relationship.

The results (table 1 (*c*)) demonstrate very clearly that cysteine in a partly "neutralized" medium (pH approx. 6) gave considerably less protection to the chromosomes than was obtained in the experiments with cysteine at lower pH-values (table 1 (*a*), (*b*)). The greatest effect of the neutralized cysteine was a 35 per cent reduction in fragment frequency, compared to the 60 per cent reduction of the pre-

vious cysteine experiments. The graphs in figure 1 clearly demonstrate this difference.

(*c*) *The Effect of Sodium Hyposulfite, $Na_2S_2O_4$:* Sodium hyposulfite was applied in the same manner as cysteine-HCl. Because $Na_2S_2O_4$ is easily oxidized, a fresh solution was substituted after the first 24 hours' exposure to radiation. As can be seen from table 1 (*d*) and figure 1 (*C*), a remarkable and significant effect was obtained even at the low concentration of 10^{-5} *M*. This series gave 12.8 fragments per 100 cells as compared to 23.4 in the control. At the stronger concentration, 10^{-4} *M*, no further decrease was obtained in the fragment frequency, 13.7 being insignificantly higher than 12.8 obtained at the previous concentration. A concentration of 10^{-3} *M* was also used, but appeared to be toxic to the roots. Thus, the maximum protection obtained with sodium hyposulfite was 46 per cent.

(*d*) *The Effect of Sodium Cyanide, NaCN:* The results with sodium cyanide, NaCN, used in two different experiments are presented in table 1 (*e*) and (*f*). Concentrations of 10^{-4} *M* and 5×10^{-4} *M* both gave 16.5 fragments per 100 cells as compared to 18.5 in the untreated control series. Thus, sodium cyanide afforded a relatively small amount of protection (about 10 per cent) at these concentrations. In another experiment (table 1 (*f*))at a concentration of 10^{-3} *M* a marked decrease in number of fragments was obtained. The fragment frequency was reduced from 22.6 per 100 cells in the control to 16.9 in the NaCN series. This significant reduction amounts to a protection of 25 per cent.

The effects of increased concentrations of sodium cyanide are also presented in figure 1 (*D*).

The Occurrence of Chromosome Bridges.—As previously indicated,[5] chromosome or chromatid reunions resulting in bridge formations at anaphase were infrequent in root tip cells following exposures to chronic irradiation. Due to a similar low frequency of bridges in the present experiments no definite conclusions can be drawn. More data relating to rejoining are required.

Discussion.—Conclusive evidence is presented for the protective ability of cysteine, sodium hyposulfite ($Na_2S_2O_4$), and sodium cyanide (NaCN) against chromosome fragmentation induced by irradiation. The mechanism, however, by which these chemicals exert their protection is not too clear. Sparrow[8] and Sparrow and Rubin[9] have discussed several factors which influence the radiosensitivity of chromosomes. Various agents which modify the genetic and cytological effects of ionizing radiations are also emphasized and the importance of an indirect radiation effect is considered. Mikaelsen[5] has emphasized the possibilities that the role of glutathione is through reduced chromosome breakage or by increased restitution of broken ends. A combination of both is a third possibility. The prevention of chromosome breakage was considered the most likely explanation.

Since the completion of these experiments Forssberg and Nybom[13] have also demonstrated a reduction in chromosome disturbances produced by x-radiation in the presence of cysteine in *Allium* roots. However, Devik[14] found no effect on chromosomal aberrations following treatments of mice with cysteine prior to x-ray exposures.

The effect of cysteine appeared to be very different in an acid and a neutral medium (table 1, Fig. 1). A similar response in the effect of cysteine, applied at different pH values, on survival of mice after whole body x-irradiation was ob-

tained by Patt, *et al.*,[15, 16] and Goldie, *et al.*[17] They state that the probable explanation of this difference may be the fairly rapid oxidation of cysteine to cystine in a neutral medium before its administration. It has been demonstrated that changes in intracellular acidity alter the radiosensitivity of the cell.[18–21] It seems justifiable, however, in this case to put the main emphasis on the oxidation process of cysteine to cystine.

Weiss,[10] Lea,[11] and others state that during exposures of water to ionizing radiation, oxidizing agents such as H_2O_2, O and the radicals OH and O_2H are formed. Since water exists in the cell nuclei, it is likely that the same reactive agents are formed in the nuclei during exposures to ionizing radiation. Barron and Flood[12] presume that these oxidizing agents are responsible for the oxidation of aqueous solutions of certain thiols by ionizing radiation, as they have demonstrated. Therefore, it seems probable that the mechanism of the sulfhydryl compound, cysteine as well as glutathione,[5] may be due to oxidizing agents produced by irradiation. These oxidizing agents may not necessarily be those mentioned above. The formation of other reactive agents (i.e., organic free radicals or organic peroxides) must also be considered.

The mechanism of the effect of sodium hyposulfite in reducing fragment frequencies is probably different from that of sulfhydryl compounds. Since sodium hyposulfite is a strong reducing agent and combines rapidly with molecular oxygen in aqueous solution, it is reasonable to assume that sodium hyposulfite decreases the amount of dissolved oxygen in the tissue. Burnett, *et al.*,[22] explained the increased survival in bacteria suspension in the presence of sodium hyposulfite during the irradiation exposure to be due to the removal of oxygen from the suspensions. By removing oxygen from the tissue, the diversion of H, for example, into the production of the secondary formation of O_2H and H_2O_2 or other reactive products is prevented. Thus, less radiochemical reactants will be formed and less breaks will be obtained. King, *et al.*,[23] claim, however, that in a large measure oxygen exerts its action by altering the biochemical processes of the cell.

In the presence of sodium hyposulfite the fragment frequency was reduced to almost half. A similar response in effect of sodium hyposulfite is obtained by Riley[24] in *Allium* roots after exposure to acute γ-irradiation. Giles and Riley[3, 4] were able to reduce the aberration frequency to one-third in Tradescantia, when exposed in vacuum or an inert gas. The difference between the effect of low-oxygen tension and sodium hyposulfite may probably be due to an incomplete removal of ozygen by sodium hyposulfite rather than to a different mechanism.

The protective ability of sodium cyanide may be more complicated and obscure than was the case with the sulfhydryl compounds and sodium hyposulfite. Bacq[25] claimed that the CN^- was responsible for the decrease in mortality that was obtained with cyanide in x-irradiated mice; the mechanism by which CN^- exerts its effect, however, is not understood. It appears, especially, as regards the two strongest concentrations, that cyanides reduce the mitotic rate. Considerably fewer anaphases (table 1) were found in these roots and the reduction in fragment frequency was small compared to the control series and the concurrent experiments with the other chemical agents. These facts indicate that metabolic inhibition or metabolic changes may be the most reasonable explanation of the protective effect of sodium cyanide. Such metabolic alterations initiated by KCN and CO are dis-

cussed by King, *et al.*,[23] and complicated enzyme systems are probably involved.

D'Amato and Gustafsson[26] have shown that cyanide (KCN) treatment of seeds increased visible mutations in barley with low concentrations of KCN. A stronger concentration 10^{-2} *M*, decreased the mutation rate, although the rate of chromosome breakage increased. These results may not be comparable to the results presented in this paper, but may indicate the complicated effects of cyanides.

Although the protective actions of these chemicals is not fully understood, evidence of an indirect effect of radiation on chromosome fragmentation is demonstrated. This indirect effect seems to be of great importance, since it is possible to reduce the radiation effect to about half in some cases (cysteine, at low pH, $Na_2S_2O_4$, glutathione[5]). Unfortunately, however, it will be difficult to determine the exact relation between direct and indirect radiation effect by chemical means, because of their toxic effects to roots at certain concentrations (table 1).

Summary.—The protective effect of cysteine, sodium hyposulfite ($Na_2S_2O_4$) and sodium cyanide against chromosome fragmentation induced by γ-irradiation is demonstrated.

The maximum effect of cysteine applied as cysteine-HCl, is a reduction in number of fragments of 60 per cent at 3×10^{-4} *M* (low pH). With "neutralized" cysteine-HCl (pH $\sim$ 6) the maximum reduction amounts only to 35 per cent at 10^{-2} *M*. The difference in protective ability between cysteine at low and high pH, is thought to be due to easy decomposition of cysteine to cystine in a neutralized medium.

The protective action of cysteine in the cell nuclei is explained by its possible reactions with oxidizing agents produced in the nuclei by irradiation.

Sodium hyposulfite ($Na_2S_2O_4$) reduces the fragment frequency 46 per cent at 10^{-5} *M*. As a strong reducing agent, it is suggested that its main effect is removal of oxygen from the tissue and that this interferes with the formation of certain reactants normally produced during irradiation in the presence of oxygen.

Sodium cyanide at 10^{-3} *M* also shows a marked protective effect and decreases the number of fragments by 25 per cent. However, the mechanism of the protective action of NaCN seems more obscure than that of the other chemical agents tested in these experiments. Induced metabolic changes may be involved.

Acknowledgment.—The author wishes to thank Dr. A. H. Sparrow, Brookhaven National Laboratory, for many valuable suggestions and advice during the course of the experiments and for assistance in preparation of the manuscript. Acknowledgment is also made to Dr. H. J. Curtis and staff members of the Biology Department at Brookhaven National Laboratory for providing facilities for this study and for many stimulating discussions.

* Research carried out at Brookhaven National Laboratory under the auspices of the U. S Atomic Energy Commission.

† Fellow of the Norwegian Research Council for Science and the Humanities, 1950–1952.

‡ Present address: Institute of Genetics and Plant Breeding, Vollebekk, Norway.

§ Also called hydrosulfite. Not to be confused with the thiosulfate ($Na_2S_2O_3$) which is also called hyposulfite.

[1] Thoday, J. M., and Read, J., *Nature*, **160**, 608–610 (1947).

[2] Hayden, B., and Smith, L., *Genetics*, **34**, 26–43 (1949).

[3] Giles, N. H., Jr., and Riley, H. P., PROC. NATL. ACAD. SCI., **35**, 640–646 (1949).

[4] Giles, N. H., Jr., and Riley, H. P., *Ibid.*, **36**, 337–344 (1950).
[5] Mikaelsen, K., *Science*, **116**, 172–174 (1952).
[6] Hoagland, D. R., and Snyder, W. C., *Proc. Am. Soc. Hort. Sci.*, **30**, 288–294 (1933).
[7] Ehrlich, M., and Fitch, S. H., *Nucleonics*, **9** (3), 5–17 (1951).
[8] Sparrow, A. H., *Ann. N. Y. Acad. Sci.*, **51**, 1508–1540 (1951).
[9] Sparrow, A. H., and Rubin, B. A., *Survey Biol. Progr.* **2**, 1–52 (1952).
[10] Weiss, J., *Brit. J. Radiol. Suppl.*, **1**, 56–59 (1947).
[11] Lea, D. E., *Ibid.*, **1**, 59–63 (1947).
[12] Barron, E. S., and Flood, V., *J. Gen. Physiol.*, **33**, 229–241 (1950).
[13] Forssberg, A., and Nybom, N., *Physiol. Plant.*, **6**, 78–95 (1953).
[14] Devik, F., *Brit. J. Radiol.*, **27**, 481–484 (1952).
[15] Patt, H. M., Tyree, E. B., Straube, R. L., and Smith, D. E., *Science*, **110**, 213–214 (1949).
[16] Patt, H. M., Smith, D. E., Tyree, E. B., and Straube, R. L., *Proc. Soc. Exptl. Biol. Med.*, **73**, 18–21 (1950).
[17] Goldie, H., Tarleton, G. T., Jr., and Hahn, P. F., *Ibid.*, **77**, 790–794 (1951).
[18] Zirkle, R. E., *Am. J. Roentgenol.*, **35**, 230–237 (1936).
[19] Zirkle, R. E., *J. Cell. Comp. Physiol.*, **16**, 301–311 (1940).
[20] Zirkle, R. E., *Ibid.*, **17**, 65–70 (1941).
[21] Marshak, A., *Proc. Soc. Exptl. Biol. Med.*, **39**, 194–198 (1938).
[22] Burnett, W. T., Jr., Stapleton, G. E., Morse, M. L., and Hollaender, A., *Ibid.*, **77**, 636–638 (1951).
[23] King, E., Schneiderman, H., and Sax, K., PROC. NATL. ACAD. SCI., **38**, 34–43 (1952).
[24] Riley, H. P., *Rec., Genetics Soc. Am.*, No. 21, 62–63 (1952).
[25] Bacq, Z. M., *Science*, **111**, 356 (1950).
[26] D'Amato, F., and Gustafsson, A., *Hereditas*, **34**, 181–192 (1948).

---

*THE DELAYED APPEARANCE OF MUTANTS IN BACTERIAL CULTURES**

BY FRANCIS J. RYAN

DEPARTMENT OF ZOOLOGY, COLUMBIA UNIVERSITY, NEW YORK

Communicated by Franz Schrader, January 9, 1954

In the course of an examination of the possibility of a delay in the growth of mutant clones because of the need for segregation of mutant from non-mutant nuclei,[1] experiments of the following sort were carried out. A high proportion of new mutants was produced by the use of ultra-violet light. The irradiated culture was spread on the surfaces of several plates consisting of an agar medium on which only mutants could form colonies. At various times thereafter the surfaces of some of the plates were respread. The number of colonies resulting would be expected to increase only if the spreading occurred after the newly arisen mutant nuclei had been separated by cell division from the non-mutated nuclei in the originally multinucleate bacteria. The delay in onset of division of the new mutants was found to be far too long to be accounted for by the hypothesis of nuclear segregation. In fact in some cases it was more than twenty-five times as long as that of the parental bacteria and of old mutants which had been irradiated and mixed on the same plates. The differential sensitivity of the new mutants requires explanation and has a direct bearing on the problem of the delayed appearance of mutants that has been observed by others.[2]