TECHNICAL PAPERS

Cytogenetic Effects in Corn Exposed to Atomic Bomb Ionizing Radiation at Bikini

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The biological experiments conducted at Bikini by the Naval Medical Research Section, Operation Crossroads, included tests of the effects of the ionizing radiation on both plants and animals.[1] Plants could be used advantageously in the genetic experiments, since large numbers of individuals are obtainable from small quantities of seeds which could be transported readily to and from the target area, and the plants could be grown wherever desired for observation and cytogenetic analysis. Dry seeds tolerate heavy doses of radiations such as gamma rays and X-rays without loss of viability and may be used to determine the biological effects of high intensities of ionizing radiation.

It must be realized that the genetic changes to be reported here are not directly applicable to man, since the amount of radiation received by the seeds would be lethal to man or animals. Corn was selected for these studies because of its tolerance for heavy doses of ionizing radiation and its suitability for both cytological and genetic studies of radiation effects, and because of the wealth of available information concerning its chromosome mechanism of heredity.

The effective radiations of the atomic bomb were compared with known doses of X-rays with respect to their action in causing direct injury to the seed and the plants grown from the seeds, and in producing visible chromosomal alterations. The heritability of the induced alterations causing pollen sterility also was studied. The samples of corn, chosen because of their uniform genetic constitution and suitability for cytological analysis of their pachytene chromosomes, included a commercial dent first-generation hybrid of two inbred lines (L289 × I205) and an inbred line of sweet corn (P51).

The corn seed was thoroughly air dried, placed in 25 moisture-proof packets, each containing from 1,500 to 2,500 seeds of the two kinds, and enclosed in canvas rolls before being placed aboard the *S. S. Burleson* in charge of Joint Task Force One of the Navy for transfer to Bikini. The seed packets were distributed on 22 ships in the target area ranging in distances up to 1,500 yards from the target ship. The placement of the seed protected them from thermal radiation, air blast, and contamination by fission products other than ionizing radiations generated by the explosion of the bomb. Control samples were retained aboard the *Burleson* outside the target area and at Beltsville, Maryland. The explosion of the atomic bomb to which the seeds were subjected took place on July 1, 1946.[2] Following the explosion the Bikini samples were returned to Washington by air express.

The design of the experiments included a comparison of the effects of the radiations from the bomb with measured doses of X-ray. Additional samples of the same kinds of corn that were used in the Bikini test were irradiated within a few days of the time the bomb was exploded with unfiltered X-rays from a tungsten target tube operated at 80 KVP. Doses of 5,000, 10,000, 15,000, 20,000, and 25,000 r were applied to the seeds at the rate of 1,000 r/min, approximately 2,000 seeds being included in each sample.

Test plantings of the bombed and X-rayed seed were made at Beltsville as soon as the samples arrived from Bikini. The plants were inspected in the seedling stage on July 29. Field plantings of selected samples which included untreated controls were made immediately thereafter at the Experimental Farm of the California Institute of Technology in Arcadia, California.

The germinability of the seed was not affected by the radiations from the bomb or by the X-ray treatments utilized in these experiments. The growth of the plants from the bombed sample nearest the target (sample A) and from X-rayed samples treated with 10,000 and 15,000 r was noticeably retarded in the seedling stage; the seedling leaves were mottled and streaked with chlorotic areas. Sectors of defective tissue occurred in the older plants, of which from 5 to 10% were deficient in growth at maturity. These effects were accentuated by the heavier doses of X-rays. Plants grown from the untreated control samples exhibited the uniform growth habit characteristic of inbred lines and hybrids.

Plants that were mottled in the seedling stage had three distinct types of visible sectors in the older leaves of the nearly mature plants which were readily classified as (1) chlorophyll deficiencies, (2) morphological anomalies, including twisted, crinkled, diminutive, or otherwise deformed leaves, and (3) dead tissue, which often resulted in a longitudinal slitting of the leaves. The observed

[1] Capt. R. H. Draeger, Officer in Charge of the Naval Medical Research Station, M. T. Jenkins, and L. J. Stadler assisted in planning the experiments reported here, and the assistance of E. G. Anderson and K. L. Retherford in growing the plants and collecting the data on plant sectors is gratefully acknowledged. Cooperating agencies included the Division of Cereal Crops and Diseases, Agricultural Research Administration, U. S. Department of Agriculture; the Department of Botany, Cornell University; and the Division of Biology, California Institute of Technology.

[2] For additional details concerning the conduct of the Biological experiments, see R. H. Draeger and Shields Warren. *U. S. Nav. med. Bull.*, 1947, **47**, 219–225.

frequencies of these types of sectors in the Bikini A and 15,000-r cultures were as follows:

The total number of plants examined was 191 from the Bikini A culture and 93 from the culture given 15,000 r

Sample	Chlorophyll deficiencies	Morphological abnormalities	Dead tissue	Plants examined	Sectors	Sectors per plant
15,000 r	68	22	20	249	110	0.44
Bikini A	126	19	73	320	218	0.60

There were fewer sectors in the cultures grown from bombed samples at greater distances from the target and those exposed to lower doses of X-rays.

The data on visible plant sectors were taken as the tassels emerged and the plants were approaching morphological maturity. The survey included only the leaves formed by the shoot apex after the seed germinated. The sectors that were recorded varied in length from approximately 50 mm to the entire length of the leaf and leaf sheath, and in width, ordinarily from 2 to 15 mm. The width of the sectors very rarely exceeded one-quarter of the width of the leaf, the larger sectors being found most frequently in the larger leaves in the region of the functional ear shoot. The prevalence of relatively small sectors in the nearly mature plant may have been due either to delayed action of the radiation or to the persistence in the region of the shoot apex of cell initials that were present in the seed. In the cytological studies of chromosomal aberrations, the sectors which were detected in the tassels ordinarily affected less than one-fifth of the tassel branches and a corresponding portion of the main spike. These tassel sectors were comparable in size to those present in the upper leaves.

There were pronounced differences in the relative frequency of the different kinds of sectors affecting the leaves in the bombed and X-rayed samples: morphological abnormalities occurred with about the same frequency, but chlorophyll deficient sectors and sectors of dead tissue were relatively much more frequent in the bombed sample than in the X-rayed sample. The cause of this selective action of the radiations from the bomb is not known.

The cytological determination of the frequency of tassel branches with visibly unaltered or normal (N) chromosomes and altered or abnormal (Abn) chromosomes involving reciprocal translocations (T), inversions (I), and deletions (D) was made at the pachytene and later stages of the first meiotic division in the microsporocytes of unselected plants of the 15,000-r and Bikini A samples. The data below were obtained from acetocarmine smears prepared from samples including about one-third of the lateral tassel branches of individual plants, the remainder of the tassel being left for pollen analysis.[3]

	Pachytene			Diakinesis			Anaphase			Type of abnormality		
Sample	N	Abn	% Abn	N	Abn	% Abn	N	Abn	% Abn	T	I	D
15,000 r	184	69	37.5	340	84	24.7	374	2	0.6	44	8	7
Bikini A	437	98	22.4	721	100	13.8	387	3	0.8	61	15	13

[3] The results of the pollen analysis of these and other plants grown from the Bikini and X-rayed samples of seed will be published by E. G. Anderson and collaborators.

of X-rays. Reciprocal translocation was the most prevalent type of induced chromosomal alteration, the total number observed being 5 times the number of deletions and inversions.

The same kinds of visible chromosomal alterations were induced by the atomic bomb and X-ray treatment. A somewhat higher frequency of alterations was observed at the pachytene and diakinesis stages in the X-rayed sample given 15,000 r than in the Bikini A sample; otherwise, the observed frequencies and kinds of cytological effects were very similar. Identical types of translocations, inversions, and deletions were observed with essentially the same relative frequency in the plants grown from the bombed and X-rayed seed.

The various types of observed chromosomal alterations induced by the radiations in these experiments are known to cause partial sterility. Thus, it was possible to determine their frequency of transmission simply by inspection of the pollen in the progeny of the irradiated plants. However, the different kinds of chromosomal alterations observed cytologically in these experiments could not be identified merely by looking at the pollen. For an analysis of transmission frequencies the plants in which chromosomal alterations occurred were outcrossed to untreated or very lightly treated plants. The frequency of plants in the F_1 progenies from the Bikini A and 15,000-r samples which exhibited pollen abortion was as follows:

	Plants examined	Plants with abnormal pollen	Per cent
15,000 r	769	56	7.3
Bikini A	870	60	6.9

In both series approximately 50% pollen abortion characterized most of the plants classed as having abnormal pollen; a few plants in both series had somewhat less than 50%, and a few had somewhat more than 50% of abnormal pollen. The data included pollen examinations of 24 progenies in the X-rayed series and 41 in the bombed series.

It may be concluded from these experiments that, with respect to the kinds and frequencies of induced chromosomal alterations, the radiations from the atomic bomb produced effects in corn comparable to those induced by

exposure to approximately 15,000 r of X-rays. Both sources of ionizing radiations produced similar phenotypic effects in the plants grown from the irradiated seed, but the bomb produced relatively more chlorophyll deficiencies and dead tissue occurring as sectors than did the treatments with X-rays.

Crystalline Human Myoglobin: Some Physicochemical Properties and Chemical Composition

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Most of the researches on myoglobin refer to the horse (*2, 3, 4, 8*), the ox (*2, 6*), and the pig (*5*). In 1947 the writer (*7*) succeeded in obtaining from human skeletal muscles pure crystallized myoglobin (1–2 gr), utilizing a procedure previously described for animal myoglobin (*6*).

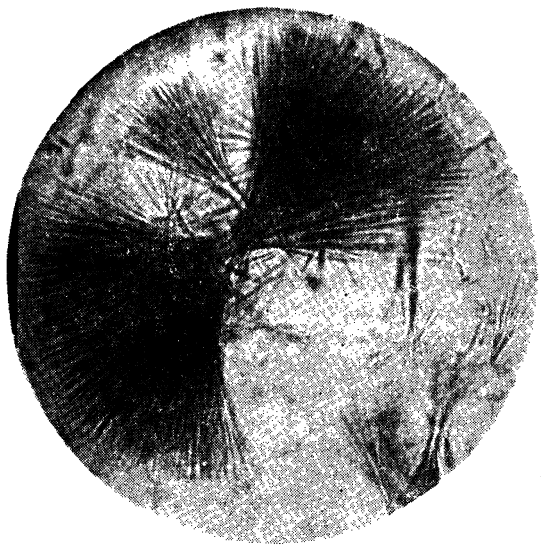

FIG. 1. Crystals of myoglobin prepared from human skeletal muscle (×370).

Human myoglobin crystals consist of long, very thin needles, tied together in subparallel bundles or in radiated, fibrous spheroidal masses (Fig. 1). The lengthening of the crystals is negative, parallel to α. Extinction is at right angles.

Crystals of metmyoglobin are clearly double-refracting, and they show an evident pleochroism with α′ of a reddish-brown color parallel to the lengthening and γ′ of a pale yellow color perpendicular to the lengthening; the refractive index is greater than 1.514. The iron content is 0.34%, the prosthetic group is probably the same as for hemoglobin, and the N content is 16.5%.

Spectrophotometric determinations gave maxima of 5,815 A and 5,426 A for the α and β absorption bands of oxymyoglobin, respectively.

The ratio between the absorption coefficients at the two maxima for myoglobin is different from that for hemoglobin. Myoglobin is comparatively stable in an alkaline medium. The formation of hemochromogen in alkaline reducing solutions may be followed spectrophotometrically. While in 0.4 N NaOH human oxyhemoglobin is rapidly transformed to hemochromogen; the oxymyoglobin still presents the two typical α and β bands of oxymyoglobin. To obtain, in this case, hemochromogen from myoglobin, the strength of the NaOH solution has to be increased to 3 N. Myoglobins from different animals (horse, ox) behave differently toward alkali—another distinguishing feature between myoglobin and hemoglobin which is, very probably, due to the chemically different composition of the two globins.

TABLE 1

	Nitrogen			
	Hemoglobin (mg)	Myoglobin (mg)	Hemoglobin (%)	Myoglobin (%)
Amide N	1.79	1.95	5.86	6.57
Humin N	1.20	1.38	3.93	4.66
Cystine N	0.153	0.138	0.50	0.47
Arginine N	2.40	1.18	7.86	3.98
Histidine N	4.08	4.10	13.37	13.85
Lysine N	2.85	3.88	9.34	13.10
Filtrate NH_2 N (monoamino acids)	16.63	15.32	54.52	51.75
Filtrate non-NH_2 N (imino acids + ½ N tryptophane)	1.26	1.50	4.13	5.06
Total N recovered	30.36	29.44	99.55	99.44

Chemical determinations carried out with myo- and hemoglobin emphasize this difference. The nitrogen distribution and the amino acid composition have been determined on 184 mg of pure crystallized human myoglobin and 186 mg of human hemoglobin prepared in the microcrystallized form (Drabkin) by a micromodification (*3*) of the Van Slyke procedure (*10*). The nitrogen distribution in human hemoglobin and myoglobin is shown in Table 1.

TABLE 2

Amino acids	Hemoglobin	Myoglobin
Cystine	0.71	0.65
Arginine	4.00	1.98
Histidine	8.09	8.22
Lysine	7.98	11.00

In Table 2, percentages of some amino acids expressed as g% of the total amount of protein are given for both proteins.

These results represent the first contribution to the knowledge of the chemical constitution of human myoglobin. As may be seen from the analytical data (Tables 1 and 2), the chemical composition of human myoglobin is different from that of hemoglobin. The most conspicuous differences are observed in the arginine and lysine content and also in the monoamino acids.