From data thus far obtained, it appears that most of the outcross plants carried one or more recessive mutations from the irradiated parent. Thus more than 50 per cent of the functioning gametes of plants from seed exposed to 15,000 r units x-ray or to the nearly equivalent atomic radiation exposure carry one or more gene mutations caused by the radiations.

The dormant seeds in these tests were exposed to much greater irradiation than the lethal dose for most animals or for actively growing plant tissue. But roughly similar frequencies obtained from 15,000 r x-ray and the nearly equivalent Bikini lot support the expectation that atomic bomb radiations have about the same heritable effects as comparable doses of x-rays.

Summary.—Maize seed receiving atomic bomb radiations equivalent to nearly 15,000 r units x-ray were tested for hereditary chromosomal alterations and gene mutations.

The frequency of chromosomal alterations as indicated by partial pollen sterility was 4.7–6.4 per cent in progenies which were not entirely random. It is estimated that a random frequency would be between 4.5 and 5.0 per cent.

The frequency of gene mutation was extremely high. Probably more than 50 per cent of the gametes of the exposed generation carried one or more gene mutations.

The frequencies of chromosomal alterations and gene mutations in a lot exposed to 15,000 r units x-ray were roughly equivalent to the frequencies obtained in the Bikini lot.

* Coöperative investigations of the Kerckhoff Laboratories of Biology, California Institute of Technology, and the Division of Cereal Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, U. S. Department of Agriculture.

This is a brief report based on a coöperative program carried on at the California Institute of Technology by a group of investigators including Dr. A. E. Longley of the United States Department of Agriculture, Prof. E. F. Frolik of the University of Nebraska, Dr. E. E. Dale of Union College and the following graduate student assistants: C. H. Li, K. L. Retherford, Earl P. Patterson, Wayne F. Keim and Don Robertson.

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*THE RELATION BETWEEN NICOTINIC ACID AND CARBOHYDRATES IN A SERIES OF MAIZE ENDOSPERM GENOTYPES**

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It has been shown by Burkholder, McVeigh and Moyer,[1] by Barton-Wright,[2] and by Mather and Barton-Wright[3] that kernels of surgary maize are about twice as high in nicotinic acid as those of starchy maize. In the first-mentioned paper, kernels of a group of sugary strains were reported to average 34.6 micrograms of nicotinic acid per gram of tissue, as contrasted with 20.8 for starchy strains; in the last two papers the averages were 26.2 for sugary strains and 15.8 for starchy. The hereditary differences in the carbohydrates in all these strains were presumably controlled by the allelic genes Su_1 and su_1, which act in such a way that kernels homozygous for the recessive su_1 are sugary, while those carrying one or more doses of Su_1 are starchy. No studies of the products of controlled crossing between Su_1 and su_1 strains were reported, but Mather and Barton-Wright[3] analyzed starchy and sugary kernels from open pollination on ears of five sugary lines. These kernels, whose triploid endosperms were of the genotypes $Su_1su_1su_1$ and $su_1su_1su_1$, respectively, showed essentially the same difference in nicotinic acid content as that cited above. (Triploidy is normal in the maize endosperm, owing to fusion of two female polar nuclei with one male gamete.)

Cameron[4] studied the effects of two pairs of alleles, su_1^{am} su and Du du, on the reserve carbohydrates in a series of maize endosperm genotypes. The gene su_1^{am} is a member of the Su_1su_1 series (for convenience, subscript numerals for this allelic series are omitted hereafter); whereas Du and du are wild type and recessive forms at an independent locus.[5] These genes interact so that endosperms carrying one or more doses of both su^{am} and Du are primarily starchy type (opaque and smooth) in appearance, while those homozygous for su or du, or both, are sugary type (translucent and wrinkled). Chemical analyses, which provide a more sensitive measure of the genetic effects, showed that the homozygous double-recessive genotype *su su su du du du* was the lowest in starch and the highest in soluble carbohydrates and that starch increased and soluble carbohydrates decreased in a rather definite pattern with successive doses of either su^{am} or Du. First doses were usually the most effective, su^{am} was more effective than Du, and one dose of each gene was usually more effective than three doses of either alone. The behavior of the entire series of genotypes with respect to starch may be seen in table 1. Notations in the dosage column of this table make clear the identity of each genotype in terms of its gene dosage: 1–0 for example, carries one dose of su^{am} and none of Du, and therefore two doses of su and three of du.

The present paper reports data on the nicotinic acid content of this genotypic series, together with exploratory data on the thiamin and biotin content of selected genotypes.

Methods.—Mature maize endosperms from the same or sister ears of the series which had served for the carbohydrate studies[4] were used for the present analyses. The ears were produced in a single plot in one season,

TABLE 1

NICOTINIC ACID AND STARCH IN MATURE MAIZE ENDOSPERM GENOTYPES RESULTING FROM COMBINATIONS OF THE TWO GENE PAIRS su^{am} *su* AND *Du du*

GENERAL PHENOTYPIC CLASS	GENOTYPE	GENE DOSAGE, $su^{am}Du$	PER CENT STARCH* (DRY-WEIGHT BASIS)	MICROGRAMS NICOTINIC ACID PER GRAM OF TISSUE (AIR-DRIED): INDIVIDUAL GENOTYPE	SUBGROUP MEAN	GROUP MEAN
Primarily starchy (opaque and smooth)						
	$su^{am}su^{am}su^{am}$ *DuDuDu*	3–3	80.7	21.7		
	$su^{am}su^{am}su^{am}$ *DuDudu*	3–2	77.7	22.3	22.2	
	$su^{am}su^{am}su$ *DuDuDu*	2–3	76.0	21.4		
	$su^{am}su^{am}su$ *DuDudu*	2–2	79.6	23.5		
	$su^{am}su^{am}su^{am}$ *Dududu*	3–1	72.7	22.4		24.2
	$su^{am}su^{am}su$ *Dududu*	2–1	71.8	29.4		
	$su^{am}su$ *su* *DuDuDu*	1–3	69.7	22.2	25.8	
	$su^{am}su$ *su* *DuDudu*	1–2	70.3	25.5		
	$su^{am}su$ *su* *Dududu*	1–1	58.6	29.4		
Sugary (translucent and wrinkled)						
	$su^{am}su^{am}su^{am}$ *dududu*	3–0	51.5	44.3		
	$su^{am}su^{am}su$ *dududu*	2–0	42.0	46.2	47.4	
	$su^{am}su$ *su* *dududu*	1–0	33.6	51.6		
	su su su *DuDuDu*	0–3	32.2	56.3		50.5
	su su su *DuDudu*	0–2	30.0	48.2	51.6	
	su su su *Dududu*	0–1	27.2	50.4		
	su su su *dududu*	0–0	19.2	56.7	56.7	

* Data from Cameron.[4]

with the exception of the genotype su^{am} *su su du du du*, which was grown in a different year.

Vitamin values are reported on an air-dry basis. Since earlier measurements had shown the entire range in moisture content to be from 7.6 to 10.5%, little error in relative values is introduced. Endosperms from each of two ears of each genotype were pooled and ground to 60 mesh in a Wiley electric mill, and the resulting material was thoroughly mixed. For the nicotinic acid assays reported in table 1, 1.0 g. of each sample was hydrolyzed by autoclaving with 50 cc. of 1 *N* sulfuric acid for 30 minutes at 15 lbs. pressure, and the suspensions were neutralized, filtered and made up to volume. The growth of *Lactobacillus arabinosus*, as determined by acid production during 72 hours at 35°C., was taken as a measure of the nicotinic acid. The culture medium and the general procedure were those described by The Association of Vitamin Chemists, Inc.[6] Calculations of nicotinic acid were made from the averages of three or more assays (each in duplicate) which agreed within 10% of the total average of duplicates representing six concentrations of added endosperm hydrolyzate. A sample of enriched wheat flour obtained from The Association of Vitamin Chemists, Inc., was assayed as a control in two experiments. The values obtained were within 3% of the range already reported by the Association (in personal communication to the junior author) for this material.

For the thiamin and biotin determinations certain strains of Neurospora were used, their response being measured by the dry weights of mycelial pads formed in a 72-hour growth period in liquid medium. The general techniques of culturing Neurospora strains, the means of supplying supplement and other procedures have been described.[7] Strain 18558, a thiamineless mutant which requires vitamin thiazole or intact thiamin,[8] was used for the thiamin estimations. The biotin estimation was carried out with wild type strain 1*A*, which is deficient in the ability to synthesize biotin. The maize hydrolyzates for the biotin assay were the same as those used for nicotinic acid determinations. The samples for thiamin assay were prepared by simultaneous takadiastase and papain treatment in acetate buffer, according to the method of Cheldelin, *et al.*[9] For each determination a standard curve was run in duplicate, and five levels of endosperm hydrolyzate were used. The average of all values read from the relatively straight-line portion of the curve was taken in calculating the vitamin levels.

Results.—In table 1 the nicotinic acid content of the genotypes is shown in relation to the previously determined values for starch.[4] Whereas the starch content had shown a stepwise response to gene dosage, ranging from 19.2% starch in genotype 0–0 to 80.7% in genotype 3–3, the content of nicotinic acid appears to be less sensitive. It is relatively low (mean, 24.2 micrograms per gram of tissue) in the group of nine starchy types that carried both su^{am} and Du, and higher by a factor of approximately 2 (mean, 50.5 micrograms per gram) in the group of seven types that lacked one or both of these alleles. The difference between the two means is highly significant, with a P value of less than 0.01. Within each of these two groups the variation among individual values is non-significant by the X^2 test; nevertheless, there is a marked tendency for nicotinic acid to reflect changes in genotype correlated with changes in starch content. This has been indicated in a column of subgroup means. Thus genotype 0–0 had a nicotinic acid content of 56.7 micrograms per gram of tissue; genotypes 0–1, 0–2 and 0–3 averaged 51.6 micrograms per gram; genotypes 1–0, 2–0 and 3–0 averaged 47.4, and so on. For the entire series, the correlation coefficient between starch and nicotinic acid is −0.97, with a P value of less than 0.01.

It has been shown that the apparent nicotinic acid content of cereal products is influenced by the method of extraction; thus wheat autoclaved with 0.1 N sulfuric acid assayed 37 micrograms of nicotinic acid per gram of

TABLE 2

EFFECT OF STRONG AND MILD HYDROLYSES ON THE APPARENT NICOTINIC ACID CONTENT OF FIVE MAIZE ENDOSPERM GENOTYPES

	NICOTINIC ACID RELEASED BY		
		MILD HYDROLYSIS	
GENE DOSAGE, $su^{a}mDu$	STRONG HYDROLYSIS, MICROGRAMS PER GRAM OF TISSUE	MICROGRAMS PER GRAM OF TISSUE	PER CENT OF STRONG HYDROLYSIS
3–3	21.7	15.0	69.1
1–1	29.4	19.3	65.6
3–0	44.3	29.6	66.8
0–3	56.3	28.3	50.3
0–0	56.7	39.0	68.8

tissue, but when treated with 1.5% sodium hydroxide or 3 N hydrochloric acid it assayed 72 to 75 micrograms per gram.[10] One N sulfuric acid also gives maximum nicotinic acid values in cereals,[11] and the data reported in table 1 were obtained by its use. To determine whether there might be differences among the genotypes in the proportion of nicotinic acid released by strong and mild hydrolyses, five of the genotypes were assayed after autoclaving with 0.1 N sulfuric acid. The results of these assays are presented in table 2 and compared with those from table 1. In each case the value obtained by mild hydrolysis was markedly lower than that obtained by strong hydrolysis. With the exception of genotype 0–3, the percentage of total nicotinic acid made available by this mild treatment was about the same for all, however, and this suggests that *su* and *du* exert no differential action in this respect. Without further tests it cannot be determined whether the one notably lower percentage (50.3, for genotype 0–3) has significance.

Results of exploratory assays for thiamin and biotin, carried out on the same five genotypes, are shown in table 3. These data must be considered only approximate, but they indicate that the two vitamins bear a relationship to the carbohydrate system similar to that of nicotinic acid, in that they are low in starchy types and higher in sugary ones. The biotin level found for genotype 3–3 agrees well with the value reported by Cheldelin and Williams[12] for white corn meal, but the thiamin level for this genotype is less than one-tenth as much as that reported by Van Lanen, Tanner and Pfeiffer[13] for a composite sample of yellow maize.

Discussion.—The data of Burkholder, McVeigh and Moyer,[1] together with those of Mather and Barton-Wright[3] and of the present paper, demonstrate a general correlation between sugary-type maize endosperm and relatively high nicotinic acid content. Three different gene combinations, su^{am} *du*, *su Du* and *su du*, when homozygous for their recessive members, are sugary type and high in nicotinic acid, while two combinations, *Su Du* and su^{am} *Du*, are starchy type and low in nicotinic acid. Thus

TABLE 3

THIAMIN AND BIOTIN CONTENT OF FIVE MAIZE ENDOSPERM GENOTYPES

GENE DOSAGE, $su^{a}mDu$	MICROGRAMS PER GRAM OF TISSUE THIAMIN	BIOTIN
3–3	0.18	0.07
1–1	0.37	0.09
3–0	0.42	0.14
0–3	0.98	0.13
0–0	0.88	0.15

changes involving either the *Su* su^{am} *su* series or the *Du du* pair are independently effective. Endosperm tissue from the gene combinatoin *Su du* has not been assayed, but since this is a starchy type it is probable that the nicotinic acid content would be low.

The present data, like those on the carbohydrates,[4] have been presented on a percentage basis, or its equivalent. In this genotypic series, however, about 1.37 sugary-type endosperms were required to equal the weight of 1 starchy-type. If the number of cells laid down in an endosperm were essentially the same in the two types (with the weight difference due primarily to differential amounts of stored carbohydrates), and if the production of the vitamins were a cell function unrelated to the genes in question, then sugary tissue might be expected to yield about 1.37 times as much as an equivalent weight of starchy tissue. Such a situation, however, does not account for the extent of the differences found. Studies on starchy maize hybrids[14] have shown that variation in nicotinic acid content can also occur as a function of the hybrid, and of the year and the location of the planting. In the present series these factors were not variables.

For comparable types, the nicotinic acid values reported here average higher than those of Mather and Barton-Wright;[3] they agree rather well with the starchy-type average of Burkholder, *et al.*,[1] but are higher than the sugary-type average reported by these workers. These differences are probably a function of the genetic backgrounds involved, for all these authors reported wide variation in nicotinic acid for both sugary and starchy kernels of various varieties. Their assays differed also in that they were apparently made upon whole kernels.

The mechanisms underlying the relationships between the carbohydrates and the three B vitamins are not known, and the possibilities are many. The primary action of the genes may relate either directly or indirectly to the carbohydrates or to any one or all of the vitamins.

It has been suggested[4] that in the starch-synthesizing processes certain steps relating to the action of phosphorylase are affected by *su* and *du*. If this is correct, changes in vitamin content may be secondary. This is plausible, since (*a*) vitamin content appears to be less sensitive than carbohydrate content to changes in genotype; and (*b*) the three vitamins all change in the same direction, as if in response to some general influence. The degree of starch accumulation might constitute such an influence. In starchy endosperms the cells become primarily storehouses for starch grains, and the production of other substances may be limited as a result.

In microorganisms shunt mechanisms exist,[15] which, although often entirely inoperative in the presence of low concentrations of (soluble) carbohydrate, form substantial quantities of certain metabolic products when carbohydrate is plentiful. In the present case the sugary maize types, in which soluble carbohydrates accumulate, may produce greater quantities of vitamins by such a mechanism. It is also possible that a portion of the vitamins or some of their components are used up (from an otherwise constant store) in the process of polysaccharide synthesis, so that smaller amounts remain in starchy kernels than in sugary ones. Thus nicotinamide, which is related to cozymase, may suffer loss due to "attrition" or other causes[16] in the course of this activity.

If it is the vitamins which the genes more directly control, different explanations are required. Conceivably, the greater quantities of vitamins in sugary genotypes could interfere with synthesis of starch or contribute to its breakdown. Again, in microorganisms it has been shown[17] that high vitamin levels can cause metabolic upsets.

It has been pointed out (by E. G. Anderson, in personal communication to the writers) that thiamin and perhaps precursors of other vitamins may appear in the endosperm by translocation from the leaves. This could imply control of these substances by genes in the diploid plant tissues, which would require a reconsideration of the gene dosages involved.

Data are accumulating which make possible the breeding of maize for greater nutritive value. It has been known for some time that the content of vitamin A-active carotenoids is controlled by a gene pair, Y_1 and y_1; yellow maize contains these carotenoids in direct linear proportion to the doses of Y_1 present; white maize lacks them.[18] In studies on the inheritance of oil, inbreds of high and low oil content have been obtained, and genetic effects are detectable in their hybrids;[19] the inheritance of proteins and amino acids is likewise being studied. In sweet corn, genes such as *du*, in conjunction with the common su_1, provide possibilities for the development of improved eating quality. The low nicotinic acid content of starchy maize is well known, especially because of its apparent relation to pellagra. A somewhat higher content can be obtained by selection among starchy hybrids,[14] and for purposes for which sugary types can be utilized the still higher levels represent an additional advantage.

Summary.—In a series of relatively isogenic mature maize endosperm genotypes, inversely related changes in nicotinic acid and starch content were found to be conditioned by genes at both the su_1 locus and the *du* locus. Nicotinic acid content appeared somewhat less sensitive to changes in genotype than did starch content. On a percentage basis sugary types averaged about twice as high in nicotinic acid as starchy types.

The proportion of total nicotinic acid made available by mild hydrolysis was similar in all but one of a group of contrasting genotypes tested.

Exploratory assays for thiamin and biotin indicated that their content in the endoserm, like that of nicotinic acid, is low in starchy types and higher in sugary ones.

Possible mechanisms underlying these relationships are discussed.

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A GENE-CONTROLLED REACTION IN NEUROSPORA INVOLVING THE SYNTHESIS OF PANTOTHENIC ACID

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The postulate that the gene is the ultimate agent controlling biochemical reactions is accepted by many geneticists as a working hypothesis. The evidence is based on the fact that gene changes bring about changes in biochemical reactions, and sometimes create a "genetic block" which makes it impossible for products to be formed. Furthermore, each gene is assumed to be specific for a specific type of biochemical reaction, with the corollary