thymidylate triphosphate (dTTP). Results of experiments in which reduction of labeled cytidylic acid (CMP) and guanylic acid (GMP) was coupled with the DNA polymerase system (*13*) are shown in Table 1. Hydroxyurea ($1.3 \times 10^{-3}M$) inhibited incorporation of CMP and GMP into DNA in this subcellular system by more than 90 percent. This inhibition is comparable in degree to the inhibitory effect of hydroxyurea ($1.3 \times 10^{-3}M$) on incorporation of thymidine into intact cells.

Two observations offer strong evidence against the concept that a disruption of oxidative phosphorylation is pertinent to the inhibitory effects of hydroxyurea on incorporation of ribonucleotides or thymidine into DNA. (i) An ATP-generating source was not used in the subcellular studies. (ii) 2,4-dinitrophenol, a compound known to induce a decrease in cellular ATP concentrations, inhibited incorporation of leucine but not thymidine in our cell system (*6*).

Deoxyadenylate triphosphate, deoxyguanylate triphosphate, and a metabolite of cytosine arabinoside are reported to inhibit the reduction of purine or pyrimidine ribonucleotide diphosphates, (*13, 14*). Deoxyadenosine ($10^{-4}M$) and cytosine arabinoside ($10^{-6}M$) inhibited incorporation of H^3-thymidine in our test system (*6*). Deoxyguanosine ($10^{-3}M$), however, was apparently inert; this nucleoside may be poorly phosphorylated by our strain of HeLa cells.

The cellular and subcellular data presented suggest that hydroxyurea alters cellular synthesis of DNA by interfering with ribonucleotide (diphosphate) reduction. The inhibitory effects of hydroxyurea on incorporation of thymidine into intestine, thymus, and regenerating liver in the rat (*10*) suggest that these tissues may provide a satisfactory source of enzymes for further studies.

After this report was submitted, results of the studies of Frenkel, Skinner, and Smiley became available (*15*). They observed decreased conversion of cytidylic acid to deoxycytidylic acid by subcellular extracts of bone marrow taken from rats and patients after treatment with hydroxyurea. Their data suggest that hydroxyurea inhibits the conversion of ribonucleotides to deoxyribonucleotides in vivo as well as in vitro. However, since they did not observe inhibition of incorporation of thymidine into DNA of treated rats, the relation between the effects of hydroxyurea in isolated systems and in intact animals requires further clarification.

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Catalase Hybrid Enzymes in Maize

Abstract. *In maize endosperm there are two electrophoretic variants of catalase. The variations are under genetic control, and the heterozygote shows three hybrid enzymes with mobilities intermediate between the parental enzymes. Thus, maize catalase may exist as a tetramer, and the hybrid enzymes may be formed by random association of two different catalase monomers.*

A large number of genetic enzyme variations in various organisms have been described (*1*). Isozymes (*2*) have not been studied with the same intensity in plants as in animals, but some important findings have been made concerning electrophoretic enzyme variations in maize endosperm (*3*). Of special interest are the formation of hybrid esterase enzymes and the gene dosage variations of those enzymes in the triploid endosperm demonstrable in reciprocal crosses. We have been investigating the formation of catalase hybrid enzymes in maize.

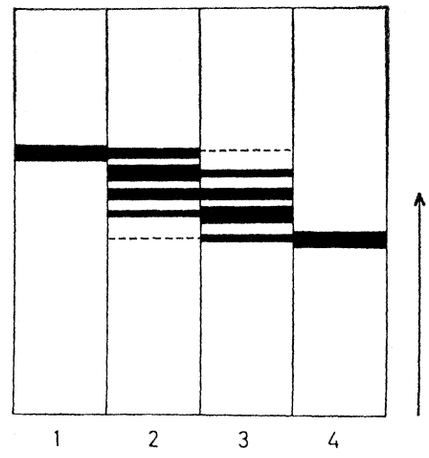

Fig. 1. Scheme of electrophoretic variations of catalase from maize endosperm; *1*, F; *2*, FH; *3*, SH; and *4*, S. The arrow shows the direction of migration toward the anode. The broken line indicates a hypothetical catalase zone that has not been visible in the zymograms. In sample *2*, the band above the dotted line, and in sample *3*, the band below the dotted line, are only occasionally visible in the zymograms.

Individual maize kernels were removed from freshly harvested ears and punctured to release the liquid endosperm, which was collected on a piece of filter paper (5×5 cm). The ears were collected on the 16th day after pollination, the time at which the liquid content of the individual kernels is almost entirely composed of endosperm. This simple method of extracting endosperm is entirely satisfactory and gives the same result as that obtained after a more complicated treatment involving homogenization and centrifugation (*3*). The filter papers were inserted into a starch gel and subjected to electrophoresis in a discontinuous buffer system (*p*H 8.6) (*4*) until the visible borate front zone had migrated about 7 cm from the sample slot. After the electrophoresis the gels were sliced horizontally and stained for catalase activity by a modification of Hale's technique (*5*). The gel was soaked first for 1 minute in 0.5 percent hydrogen

peroxide, washed twice in distilled water, and then immersed in a 1 percent solution of potassium iodide acidified with glacial acetic acid. The peroxide releases iodine which stains the starch gel dark blue except in the areas where the catalase activity has destroyed the peroxide.

Endosperm extracts from different maize stocks harvested on the 16th day after pollination show one sharply delineated zone of catalase activity. In contrast, extracts of some animal materials (HeLa cells, human erythrocytes, and homogenized whole *Drosophila* individuals) tested with the same technique invariably showed very wide, blurred zones of catalase activity. Two electrophoretically distinct catalase variants were found in different stocks. The variant with a faster mobility towards the anode was called F and the slower variant was termed S.

Crosses were made to demonstrate the inheritance of this variation (Table 1). Two inbred lines of sweet corn were used: AA^4 (University of Hawaii), with a fast band, and P39 (Purdue University), having a slow band. Self pollination of AA^4 gave only offspring of type F and self pollination of P39 yielded S offspring. The hybrids between AA^4 and P39 had multiple catalase bands (Figs. 1 and 2). There were two distinct patterns of hybrid enzymes, one with three clearly distinguishable bands of which the fastest coincided with the F band, and another pattern with three bands where the slowest band had the same mobility as the S variant. Sometimes a fourth band could be seen. The hybrid pattern with an activity concentration in the fast bands was called FH and the one with a concentration in the slow bands was called SH. The F_1 hybrids of the $AA^4 \times$ P39 ♂ cross were of the FH type, and the hybrids of the reciprocal cross were SH. Backcrosses yielded parental and hybrid types in close agreement with the expected 1 : 1 proportions with one exception: in the cross P39 × (AA^4 × P39) one offspring of type SH and 29 of S type were found, which is a significant deviation from the expected 1 : 1 ratio. Reciprocal backcrosses showed different hybrid types. In the F_2 generation there were four types F, FH, SH, and S; this is the only cross showing both hybrid types among the offspring.

Our results may be interpreted as follows: The catalase variation is controlled by two codominant alleles (Ct^F and Ct^S). Individuals homozygous for the Ct^F allele show only the fast moving catalase zone, and homozygotes for Ct^S have only the slow enzyme band. The types FH and SH are both heterozygotes (Ct^F/Ct^S). Type FH is produced when the Ct^F allele is contributed by the maternal parent and thus is present in double gene dose. A double dose of the Ct^S allele in a cross where this allele is the maternal contribution gives the SH type. The F_2 accordingly shows two types of heterozygotes. Thus, the maize catalases may exist as tetramers and the hybrid pattern could be a result of random combination of two different catalase subunits. The fastest catalase enzyme would then be composed of four F units (FFFF), the next band can be written as FFFS, the third band FFSS, the fourth FSSS, and finally the slowest band as four S-units (SSSS). In a diploid tissue the expected binomial proportions of these five possible combinations would be 1 : 4 : 6 : 4 : 1, on the assumption of random combination. The F_1 hybrids from an F × S ♂ cross would be expected to show the following proportions of the five tetramers in endosperm tissue: 16/81 FFFF, 32/81 FFFS, 24/81 FFSS, 8/81 FSSS, and 1/81 SSSS. The observed variations in strength of the catalase hybrid bands are in good agreement with the theoretical expectation (Fig. 1).

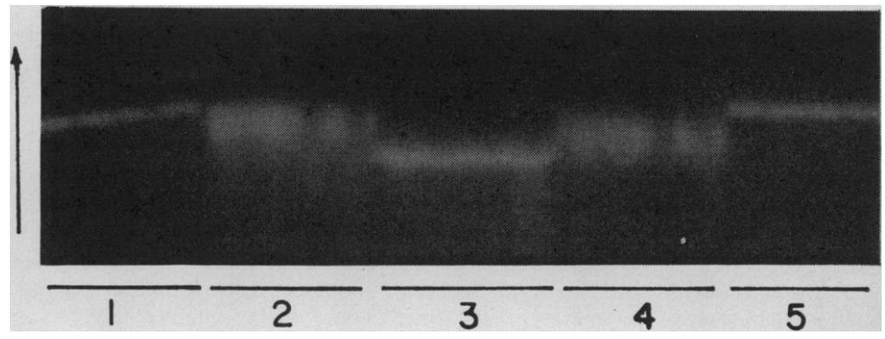

Fig. 2. Zymogram of electrophoretic variations of catalase from maize endosperm. *1*, Samples from four kernels of the AA^4 strain; *2*, samples from four kernels of the hybrid $AA^4 \times$ P39 ♂; *3*, samples from four kernels of the P39 strain; *4*, samples from four kernels of the hybrid P39 × AA^4 ♂; *5*, samples from four kernels of the AA^4 strain. The arrow shows the direction of migration toward the anode.

Table 1. Results from crosses made to demonstrate the genetic control of catalase variations in maize endosperm.

Parent		Catalase patterns of offspring				
Female	Male	F	FH	SH	S	Total
AA^4	AA^4	30	0	0	0	30
P39	P39	0	0	0	30	30
AA^4	P39	0	30	0	0	30
P39	AA^4	0	0	30	0	30
(AA^4 × P39)	AA^4	13	0	17	0	30
AA^4	(AA^4 × P39)	18	12	0	0	30
(AA^4 × P39)	P39	0	16	0	14	30
P39	(AA^4 × P39)	0	0	1	29	30
(AA^4 × P39)	(AA^4 × P39)	10	15	13	22	60

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