the low-molecular-weight region of a gel-filtration profile. The ion-exchange beads were removed by filtration, and the protein was recovered by lyophilization. The protein could be resolved into four components both by Tiselius electrophoresis and by chromatography on carboxymethyl-Sephadex with a salt gradient (the activity coefficient was 0.5 initially, and 2.0 finally; approximately linear; a *p*H 8 phosphate buffer was used throughout). Three of these fractions were radioactive and contained approximately 1,2 and 1 residue of phenol-2,4-disulfonyl chloride per molecule of lysozyme. On the basis of mobility, point of elution, and specific radioactivity it would appear that the slowest-moving peak electrophoretically, which corresponds to the first peak eluted from the carboxymethyl-Sephadex, is a dimer. The dimer (less than 10 percent of reaction mixture) contains two cross-linking residues, one of which at least is involved in an intermolecular linkage. The fourth peak was established to be unreacted lysozyme. The fractions had 25, 80, 74, and 98 percent of the enzymatic activity of the control. The presence of large amounts of dimer was ruled out by analysis of the data obtained from a Spinco Model E ultracentrifuge. The optical rotatory dispersion (ORD) spectra of the lysozyme and the cross-linked fractions gave identical rotations above 320 mμ; that there was some difference below 320 mμ may be indicative of unknown structural change.

In order to locate the positions at which the cross-linking reagent had reacted, the appropriate fractions were subjected to the sequence of reactions (*6*) that provides for the generation of sets of tryptides (peptides from a digestion by trypsin) and their separation. The determination of the amino acid composition of each fragment allowed an unambiguous assignment of the position of cross-linking to a particular residue in the sequence (Table 1). Amino acids were reported if the spot on the thin-layer chromatogram was of comparable intensity to the other spots and at the exact position of the two controls. Assignment of a particular composition to a given tryptide sequence was made on the basis that, even though the analytical data were incomplete, there was only one Canfield tryptide that contained the amino acids identified in our work, and that did not also contain several amino acids not found here. Thus the data in Table 1 indicate that three cross-links were introduced—only one in any given molecule—and these are between lysines at positions 33 and 96, 33 and 97, and between 33 and 116 [numbers in primary sequence according to Canfield (*3*)]. Most, if not all, of the ε-amino residues and perhaps the α-amino group have been sulfonylated; cross-linking can follow in some cases, and in the others none is seen to occur. Sulfonylation without cross-linking can arise from hydrolysis of one of the $-SO_2Cl$ groups before, or after, sulfonylation by the other. In addition to showing that particular amino acid residues are involved in the cross-linking, the infrared spectra of the appropriate fractions showed new absorptions characterized as arising from sulfonamide bonds (*7*).

We conclude that (i) the cross-linking has resulted in the introduction of new covalent bridges between pairs of ε-amino groups, (ii) most of the amino groups are "available" for sulfonylation even if not for cross-linking, and (iii) the cross-linking has not resulted in any substantial change in enzymatic activity. The ORD spectrum shows no measurable difference when compared with the control above 320 mμ and some between 220 and 320 mμ; taken with the enzyme-activity data it must mean that a degree of conformational integrity is retained. However, more detailed examination (*8*) reveals that the cross-links can only be formed by rearranging the side chains of lysine. This is not surprising since it would have seemed unlikely, a priori, that any reagent could match exactly the spacing requirements for any potential cross-link. Thus, a certain degree of rearranging from the crystalline structure must be expected. If the rearrangement required is too extensive (*9*), the described cross-linking process per se may be suspected of causing changes in the structure. On the other hand, a consistent pattern of cross-linking of pairs of functional groups of reagents of different chemical properties or different geometry (or both) may be presumptive evidence that the side chains of the amino acid residues are free to move when the protein is in solution.

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Durum-Type Wheat with High Bread-Making Quality

Abstract. *A durum-type wheat (2n = 4x = 28) with high bread-making quality was produced by crossing a durum-wheat variety with a common bread-wheat variety (2n = 6x = 42), backcrossing to the bread-wheat variety for three generations, and then selecting for 28-chromosome plants. The high quality is tentatively attributed to a translocation involving one of the D-genome chromosomes.*

The fact that the hexaploid wheat of agriculture (*Triticum aestivum* L. em. Thell.), genomically AABBDD, has been produced by the addition of the chromosomes of *Aegilops squarrosa* L., genomically DD, to the tetraploid emmer wheat (*Triticum dicoccoides* Korn. in litt. in Schweinf.), genomically AABB (*1*), is generally accepted. The varieties of present-day durum wheat, *T. durum* Desf., originated from emmer wheat. These two main classes of cultivated wheat differ in a number of characteristics, primarily in yield in favor of the durum wheat (*2*) and in bread-making quality in favor of the hexaploid wheat. This better bread-making quality of the hexaploid wheat has been attributed to the influence of the D-genome chromosomes, although there are conflicting reports regarding the chromosomes that carry the genes for quality (*3*). The desirability of producing a durum wheat with satis-

factory bread-making characteristics cannot be overemphasized; this is even more important due to the ever-increasing world population.

Therefore, using the method described by Kerber (*4*), we undertook a program of extracting the AABB component from a number of leading hexaploid-wheat varieties. Three varieties of *T. aestivum* L. em. Thell., namely, Thatcher, Rescue, and Prelude, were crossed to the durum variety Stewart 63, and backcrossed five, four, and three times, respectively, to the hexaploid variety. Afterward, stable lines with 14 bivalents in first metaphase were selected from each combination. The seed used for the experiments was increased for three generations in the greenhouse and then was grown in the field. Under experimental plot conditions, the extracted tetraploids yielded approximately 50 percent of their respective hexaploids. The extracted tetraploids and their parents were subjected to a large number of tests to evaluate their bread-making potential.

Results (Fig. 1 and Table 1) were obtained by standard testing methods (*5*). Definition and discussion of the significance of the various quality parameters are given in a standard text on wheat quality (*6*). In the farinograph test (Fig. 1), a flour of good quality develops into a dough of optimum consistency relatively quickly (5 to 7 minutes) and maintains most of its maximum consistency for a long mixing time. Farinograph curves for such flours reflect strong characteristics.

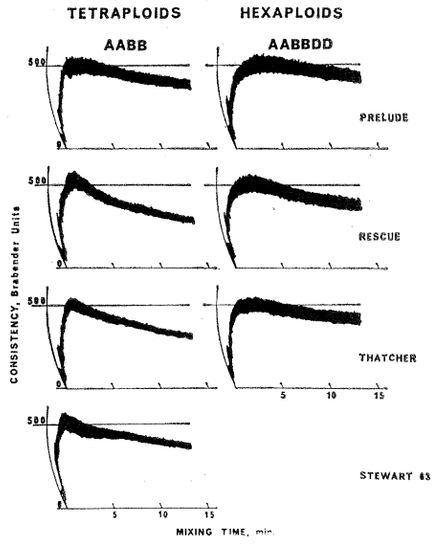

Fig. 1. Results of dough mixing. Curves were obtained with a Brabender farinograph.

Increase in all but one of the parameters (Table 1) indicates better quality; ash content of flour which depends on ash content of the wheat is usually inversely related to its bread-making quality.

In regard to dough mixing, curves for two of the tetraploids (Fig. 1) are somewhat weaker than the curve for the durum variety Stewart 63, whereas the curve for the tetraploid Prelude is somewhat stronger. In general, the farinograph curves indicate that the extracted tetraploids are weaker than their hexaploid counterparts. With one significant exception, the data in Table 1 show that the bread-making quality of extracted tetraploids is relatively poor, not too unlike that of standard durum varieties. The one exception is tetraploid Prelude, which showed superior baking quality and was comparable to that of the hexaploid wheats. Although the sedimentation test indicates that the quality of its protein for bread might be slightly inferior to that of its hexaploid counterpart, the additional protein content more than makes up for this lower quality and, in the actual baking test, yields a higher loaf volume. The high water absorption of the tetraploids is due to the extensive starch damage produced in milling the extremely hard, durum-like kernels. This factor is not reflected in the baking absorption.

The data on quality (Table 1) present additional evidence on the importance of the D-genome in the inheritance of baking quality. The somewhat higher quality of the tetraploid Prelude is attributed to two factors: (i) higher protein content and (ii) additional quality introduced genetically by a translocation involving one of the D-genome chromosomes and one chromosome from either the A- or B-genome. This hypothesis is based on the fact that, when tetraploid Prelude was crossed to its hexaploid counterpart, there was a trivalent chromosome association in the majority of the cells in the first metaphase of the pentaploid hybrid. We are attempting to identify the chromosomes involved in the translocation by crossing with the aneuploid series that exist in Chinese Spring, a hexaploid-wheat variety, and also to

Table 1. Bread-making quality of extracted AABB wheats and their AABBDD counterparts. I, AABB; II, AABBDD. Deviations from standard testing methods are indicated in footnotes. S.V., sedimentation value. Ash and protein contents are on 14 percent moisture basis. B.U., Brabender units.

Type	Wheat				Flour						Bread	
	Weight							Farinograph				
	Bushel (kg)	10^3 Kernel (g)	Protein (%)	Yield flour (%)	Ash (%)	Protein (%)	S.V.*	Absorption (%)	Devel. time (min)	Mixing tolerance (B.U.)	Baking absorp.† (%)	Loaf vol.‡ (cm^3)
					Prelude							
I	29.0	21.5	14.7	68.2	0.78	13.8	33	73.4	3.0	60	60.4	755
II	30.4	30.7	13.0	75.1	.48	12.3	54	62.1	5.0	50	57.1	730
					Rescue							
I	28.6	17.5	15.3	58.6	.94	13.8	18	82.7	2.6	165	60.1	435
II	30.4	34.9	11.4	74.6	.50	10.8	66	62.7	3.5	75	58.7	705
					Thatcher							
I	26.8	22.3	13.2	54.8	.93	12.2	18	80.2	2.5	100	60.2	470
II	30.0	31.9	13.2	74.1	.45	12.1	67	67.8	4.0	50	62.8	785
					Stewart 63							
I	30.9	56.2	11.8	66.1	.66	10.8	19	68.5	1.8	85	56.5	400

* A.A.C.C. method 56.61 (5). † Adjusted for proper dough handling at panning. ‡ Remix baking test [*Cereal Chem.* 37, 603 (1960)]. Loaf volume is judged per 100 g of flour.

transfer the translocated chromosome into a number of high-yielding durum varieties in order to produce a high-yielding durum wheat with acceptable bread-making quality. It might be feasible to extract other desirable characteristics from established hexaploid-wheat varieties that carry translocations involving the D-genome by crossing them to durum-wheat varieties and by isolating the desirable characteristics in the extracted tetraploid.

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Membrane Origin of the Fast Photovoltage of Squid Retina

Abstract. *When a bright light flash is absorbed by a small region in the outer segments of squid photoreceptors fixed in glutaraldehyde, a brief pulse of membrane current flows locally. The passive spreading of this current along the outer segments produces the photochemical component of the "early receptor potential." The source of the current lies electrically in parallel with the cell membranes and perhaps is located within them. Fixation with glutaraldehyde apparently does not reduce the resistance of the cell membrane to less than 5 percent of its value in live cells.*

Intense flashes of light produce small transretinal voltages of very short latency ("early receptor potentials") in the eyes of many different animals (*1, 2*). Both in vertebrate rods (*2*) and in squid photoreceptors (*3*), photochemical reactions of rhodopsin underlie the generation of these fast photovoltages (FPV's), but the mechanism producing electrical events from chemical reactions is not yet understood. Since drastic changes in the nature and concentration of the electrolytes bathing the retina and even fixation with glutaraldehyde or exposure to temperatures below 0°C do not abolish FPV's (*3–5*), it is generally thought that changes in the membrane permeability, such as produce ordinary electrophysiological responses like the electroretinogram, are not involved. Instead, it has been suggested that the FPV might be a direct external sign of changes in the distribution of charges in rhodopsin molecules as they undergo photochemical reactions (*4, 6*). We now report a test of this idea which leads us to conclude that the photochemical component of the FPV in squid photoreceptors is indeed a charge displacement in the electrical capacitance of the plasma membranes of the outer segments. The thermal component of the FPV previously described (*3*) is small enough in the present experiments to be negligible.

Live squid retinas were prepared as described previously (*7*), all manipulations being carried out in darkness or under infrared illumination with the aid of image converters. A retina was attached by suction to a membrane filter (Millipore Type HA) and cut into rectangular blocks (300 by 700 μm) by cuts made parallel to the long axes of the photoreceptors. A single block consisting of photoreceptor cells and all other retinal layers was fixed for 20 minutes at 0°C in 5 percent buffered glutaraldehyde in seawater. The block was then washed with cold seawater and placed on the cooled stage of an infrared microscope, with the long axes of the photoreceptors parallel to the horizontal plane of the stage. Fluid was blotted away until the block was insulated by moist air on five sides and by the supporting cover slip on the sixth. Light from a xenon flashtube was freed of wavelengths less than 520 nm and focused into a narrow beam whose cross section was a rectangle 16 μm wide and 700 μm long. The beam was directed downward through the block so that the long axis of the rectangle and the direction of propagation of the light were both normal to the long axes of the outer segments. All outer segments were thus illuminated at the same distance from their inner ends. With this arrangement, the light-induced voltage gradients in the interstitial spaces of the block were found to be negligible in all directions save that parallel to the long axes of the photoreceptors. The locations of sources and sinks along the cells could therefore be found by analyzing the voltage gradients in the context of a one-dimensional boundary-value problem.

When two micropipettes filled with seawater and connected to a differential amplifier were placed on the upper surface of the block with their tips in a line parallel to the long axes of the outer segments, the interelectrode voltage waveforms (Fig. 1) produced by repeated flashes and summed in a transient averaging computer closely resembled the externally measured FPV of the light-adapted retina (*3*). But the sizes and polarities of the responses varied according to the location of the light beam relative to the electrode pair. When the beam fell between the black pigment layer and the lower electrode, small waves whose fast phases were in the upward direction (electrode A positive) were seen. As the slit was advanced upward in steps toward the inner ends of the outer segments, the voltage difference between the pipettes first increased, then diminished and reversed in sign, growing to maximum size with the beam at A and finally diminishing as the beam approached the inner limiting membrane (ILM) at the top of the figure. The beam position where reversal of the voltage transients occurred shifted with the electrodes if the latter were moved along the block parallel to the long axes of the outer segments. Thus the pattern of voltage differences depended mainly on the relative positions of light and electrodes, not on their absolute position on the block. The same dependence of FPV form on stimulus position was also seen when the electrodes were thrust into the block, when the cell bodies of the photoreceptors were cut away from the block, or when blocks of live retina were used.

This result is not consistent with any theory which places the current generators responsible for the FPV within the bulk of the interstitial spaces; in the