in *N. rustica* × *N. tabacum* seeds in which collapse is delayed so that a germinable condition may be reached, even though very rarely, the endosperm-nucellus volume ratio at early post-fertilization is higher than in *N. rustica* × *N. glutinosa* seeds, which all fail early.[4] Since the pistillate parent is the same in both crosses, it is evident that the varying degree of abnormal development of the maternal tissue in the two respective classes of seed is a function of the endosperm genotype.

The endosperm, therefore, may act as a barrier to interspecific hybridization in flowering plants. The tissue forms the basis of an isolating mechanism, to use Dobzhansky's[5] term, which tends, like numerous other physiological devices, to hinder the interchange of genes between specific groups.

It is a point of considerable practical interest that failure of the seed due to weak endosperm development does not necessarily imply that the associated embryo is inviable. The lethal mechanism is not in the line of descent but is parallel to and impinges upon a short but critical segment of it. Freed of its adverse environment in the seed before breakdown occurs, the embryo may be capable of growing into a plant. Numerous cases are on record of vegetatively vigorous interspecific hybrids having been reared from badly shrunken seeds which may have barely passed the limits of germinability in their development. If the present analysis is well founded, it is a fair inference that below this threshold there are embryos of many more hybrids the realization of whose potentialities only awaits the application of suitable technics of artificial cultivation. The work of Laibach,[6] Tukey,[7] van Overbeek *et al.*[8] demonstrates the possibilities of rearing incompletely developed embryos on synthetic media. The limits within which plant breeders may be able to explore the possibilities of interspecific hybridization now appear, therefore, to be significantly broader than earlier could have been foreseen.

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ON THE LOCALIZATION OF ENZYMES IN NERVE FIBERS[1]

CHOLINE esterase is highly concentrated at or near the neuronal surface. This was shown recently in experiments on the giant fiber of squids, where practically the whole enzyme activity was found to be in the sheath, only a negligible amount of enzyme being present in the axoplasm.[2] These observations, together with the parallelism, within certain limits, found between number of electric plates per cm, E.M.F. per cm and concentration of choline esterase in electric organs, suggest that the electrical changes observed during nerve activity may be intrinsically connected with acetylcholine metabolism.[3,4] It is necessary to know how specific is the concentration of choline esterase at the neuronal surface compared with other enzymes important for nerve activity. Studies have been started in order to determine the activity of different enzymes separately in sheath and axoplasm of the giant fiber.

Nerve activity is connected with heat production and extra oxygen uptake. Determinations of the total respiration may not yield optimal values if the cell is no longer intact. It appears safer to determine important intermediate steps. Succinic dehydrogenase is widely believed to be essential in respiratory enzyme systems. Its activity has been determined with the manometric method with ferricyanide.[5] Table 1 gives

TABLE 1

SUCCINIC DEHYDROGENASE. K = CONTROL, E = WITH SUCCINATE. TIME 235 MIN. CONCENTRATION OF SUCCINATE 0.15 MOL. t = 23° C.

		Mg fresh tissue	Cmm CO_2		μg succinic acid		
			Observ.	Calc. for 100 mg	Absol.	In per cent. of total	Per 100 mg
Sheath	K	6.7	1.96	29.2			
	E	5.5	4.20	76.7	6.7	11	126
Axoplasm	K	27.5	4.5	16.3			
	E	24.9	24.6	99.0	53.5	89	216

the results of an experiment showing that about 90 per cent. of the total enzyme amount is present in the axoplasm. The concentration is about 50 per cent. lower in the sheath, although the absolute amount metabolized there is so small that the values are near to the limits of sensitivity of the method. Although the greatest part of the sheath is connective tissue, the results give no indication of a higher concentration of succinic dehydrogenase at or near the surface. The contrast to the distribution of choline esterase is striking. In the whole giant fiber the concentration is intermediate between that of sheath and axoplasm, as could be expected. In the head ganglion the concentration of succinic dehydrogenase is about 10 times as high as in the fiber, whereas its concentration of

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choline esterase is several hundred times higher than in the fiber.

In one experiment with succinic oxidase the enzyme distribution between sheath and axoplasm was the same as that found for succinic dehydrogenase. The difference of enzyme concentration between fiber and head ganglion was also the same.

The formation of acetylcholine occurs only in oxygen and in presence of either glucose or pyruvic acid.[6] This suggests that the acetic acid is formed from pyruvic acid, a process which probably requires vitamin B_1. Mann and Quastel found that in the brain of vitamin B_1 deficient pigeons acetylcholine formation is accelerated in presence of potassium + vitamin B_1.[7] The greatest part of vitamin B_1 in living tissue seems to occur as diphosphothiamine (cocarboxylase). The concentration of this coenzyme has been determined in sheath and axoplasm. The method used was that of Lohmann and Schuster with slight modifications.[8] Table 2 gives an experiment showing that the concentration in the sheath is twice as high as that in the axoplasm. In view of the large fraction of connective tissue in the sheath these figures definitely indicate a concentration of diphosphothiamine in or near the surface many times as high as that in the axoplasm. The distribution differs from that of choline esterase as well as from that of succinic dehydrogenase and oxidase. It supports the assumption that one function of vitamin B_1 may be its participation in the formation of acetic acid for acetylcholine.

TABLE 2

COCARBOXYLASE. TIME 20 MIN. K = 0.05 μ COCARB.

	Mg fresh tissue	Cmm CO_2	μg cocarboxylase	
			Absol.	Per 100 mg
Sheath	14.0	2.66	0.0315	*225*
Axoplasm	87.0	6.89	0.0785	*90*
K		4.39		

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

A COLOR REACTION FOR DEHYDROASCORBIC ACID USEFUL IN THE DETERMINATION OF VITAMIN C

WHEN the 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid is treated with 85 per cent. sulfuric acid, a reddish colored product is formed which absorbs maximally at 500–550 mu and 350–380 mu. The nature of this reaction is illustrated by the following experiment. One cc of a dilute acetic acid solution of dehydroascorbic acid is mixed with one cc of a saturated solution of 2,4-dinitrophenylhydrazine in 85 per cent. H_3PO_4. After letting stand for 5 minutes, 8 cc of concentrated H_2SO_4 is added. The same red color is obtained as that observed when the crystalline 2,4-dinitrophenylhydrazine derivative of dehydroascorbic acid is dissolved in H_2SO_4. If more time is allowed for the 2,4-dinitrophenylhydrazine to couple with the dehydroascorbic acid, a deeper red color is obtained when H_2SO_4 is added. If the three substances involved in this reaction are mixed in any other sequence, the red color is not obtained. Hence, this reaction is due to the action of H_2SO_4 on the coupled 2,4-dinitrophenylhydrazine-dehydroascorbic acid compound.

The proportionality of the color obtained in this reaction is in good agreement with Beer's law. We have used this reaction to develop a simple and rapid colorimetric method for the determination of ascorbic acid. Possible interfering substances are pentoses, glucose and fructose, which in concentrations much greater than usually found in acid extracts of plant and animal tissues will form derivatives with 2,4-dinitrophenylhydrazine. The latter dissolve in 85 per cent. sulfuric acid, giving brown to yellow colors, but they do not react with the acid except when heated or upon long standing. Furthermore, the absorption curves of the sulfuric acid solutions of the xylose, glucose and fructose derivatives of 2,4-dinitrophenylhydrazine, and also of 2,4-dinitrophenylhydrazine, show practically complete transmission in the wave-lengths at which the red color obtained from dehydroascorbic acid is compared. Hence, it appears that a completely specific principle for the determination of ascorbic acid is offered.

In the proposed method, the 2,4-dinitrophenylhydrazine derivative is isolated from unknown and standard solutions of ascorbic acid according to published directions[1] and treated with 85 per cent. sulfuric acid. Colorimetric comparison is made, using a filter with maximum transmission at 540 mu. The method will be published in detail later.

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