Table 1. Oxygen uptake by excised goldfish gills in succinate, sucrose, and succinate plus mercuric chloride substrates (microliter of O_2 per milligram of dry weight of gills).

Intervals	Succinate substrate (avg. of 35 fish ± S.E.$_m$)	Series I: Sucrose substrate (avg. of 11 fish ± S.E.$_m$)	Series II: Succinate plus $10^{-5}M$ $HgCl_2$ substrate (avg. of 12 fish ± S.E.$_m$)	Series III: Succinate plus $10^{-3}M$ $HgCl_2$ substrate (avg. of 12 fish ± S.E.$_m$)
30 min	1.99 ± 0.21	0.23 ± 0.14	2.20 ± 0.32	1.18 ± 0.14
60 min	3.86 ± 0.22	.41 ± 0.14	3.71 ± 0.42	1.87 ± 0.21
90 min	5.53 ± 0.26	.71 ± 0.26	5.09 ± 0.50	2.24 ± 0.22
"P" for 90-min interval		< .001	0.297	< 0.001

Thirty-five goldfish obtained from a commercial hatchery were used in these experiments. Filaments stripped from excised gill arches were used for experimental tissues. Each fish served as its own control. The Warburg apparatus was used to determine succinic dehydrogenase activity by the method suggested by Umbreit, Burris, and Stauffer (*5*). Three milliliters of substrate were used in each flask.

In series I, sucrose was substituted for sodium succinate as a substrate. In series II and III, sodium succinate was used as a substrate. In series II, mercuric chloride was made up in succinate substrate and placed in the side arm of the flask. Its concentration was such that when tipped into the flask it produced a concentration of $10^{-5}M$ mercuric chloride for the entire substrate. The same procedure was followed for series III except that the final concentration of mercuric chloride in the substrate was $10^{-3}M$. In series II and III, unaltered succinate substrate was tipped into the control flasks. After 20 min for equilibration, readings were taken at 0-, 30-, 60- and 90-min intervals. All tip-ins were made at the 30-min interval. Calculations are based on the percentage dry weight of the gill tissue.

The results of these experiments are presented in Table 1. The first column gives the average oxygen uptake for all gills run in the pure succinate substrate. The "P" values presented are for the 90-min interval and compare the oxygen uptake in the succinate substrate with oxygen uptake in the sucrose substrate, succinate plus $10^{-5}M$ mercuric chloride substrate, and succinate plus $10^{-3}M$ mercuric chloride substrate, respectively.

The significantly greater uptake of oxygen by gill filaments in a succinate substrate (5.53 μlit of O_2 per milligram of dry weight) as compared with the uptake in sucrose substrate (0.71 μlit of O_2 per milligram of dry weight) at the 90-min interval is considered proof of the presence of succinic dehydrogenase in the gills of goldfish. According to Barron and Kalnitzky (*6*), mercuric chloride inhibits the activity of this system by combining with essential sulfhydryl groups. Such an inhibition was found to occur when $10^{-3}M$ mercuric chloride was added to the substrate (series III). The uptake of oxygen was reduced 59.5 percent. Since Meyer (*7*) has inhibited active uptake of sodium in the goldfish gill with mercuric chloride, it is suggested that this work offers additional evidence in favor of the theory that succinic dehydrogenase is involved in active sodium transportation.

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Determination and Inheritance of Nicotine to Nornicotine Conversion in Tobacco

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The presence of a genetic factor in certain low-nicotine strains of tobacco controlling conversion of nicotine to nornicotine during air curing has been reported by Valleau (*1*). The change of a portion of the nicotine to nornicotine reduces the alkaloid content of the smoke, since the transfer of nornicotine into the smoke is less than one-fourth of the transfer for nicotine (*2*). Utilization of this genetic factor in commercial varieties could be advantageous, since, with acreage control, overfertilization of burley tobacco, in an effort to increase yields, has tended to raise the nicotine content of some crops to an undesirable level.

The development of the following paper chromatography method, based in part on the work of others (*3*), has permitted extensive investigations (*4*). In this method, 1 g of a finely ground tobacco sample was placed in a 15-ml centrifuge tube. Five milliliters

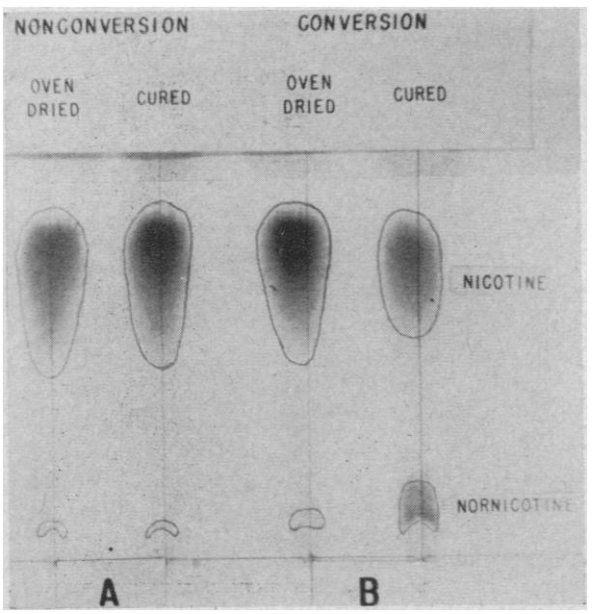

Fig. 1. Chromatogram of oven-dried and cured samples from conversion and nonconversion plants.

of an extracting solution (chloroform-methanol-water, 15 : 9 : 1 by volume, saturated with sodium hydroxide) was added and the mixture was stirred thoroughly. After 30 min, the stirring was repeated and the samples were centrifuged. Ten microliters of each extract was spotted at 2-cm intervals, 2 cm from one edge, on 23- by 28-cm pieces of Whatman No. 1 filter paper that had been impregnated with a 0.2*M* citric acid solution adjusted to a *p*H of 5.65 with sodium hydroxide. The chromatograms were developed overnight with tertiary amyl alcohol saturated with water and β-naphthylamine, using an ascending technique. Before being dried, the chromatograms were placed in an acetic acid atmosphere. After drying they were briefly exposed to ammonia and then placed in a cyanogen bromide vapor chamber for color formation. The alkaloids appeared as brightly colored orange and red spots. The Rf value of nicotine was 0.55 and of nornicotine 0.11.

Representative chromatograms of nonconversion and conversion plants are illustrated in Fig. 1. Little, if any, change in nicotine or nornicotine occurs during air-curing of nonconversion strains (*A*). The nicotine spots of air-cured samples of conversion lines are smaller and less intense and the nornicotine spots are larger and more intense (*B*) than the comparable oven-dried samples, indicating that during curing nicotine was changed to nornicotine.

Previous segregation data of a heterozygous line indicated that conversion was controlled by a single dominant gene. Additional data were obtained from crosses made between a low-nicotine true-breeding conversion line and a true-breeding nonconversion high-nicotine line. The F_1 generation was grown in the greenhouse and backcrossed to the parent lines. The F_2 generation and backcross plants were grown in the field. Samples consisting of four leaves from each plant were taken from the greenhouse plants at maturity and from young field plants. Two leaves were immediately dried at 70°C in a forced-draft oven and the other two were air cured.

The results obtained when progenies were tested for conversion are given in Table 1. These results furnish further proof that conversion of nicotine to nornicotine during curing is controlled by a single dominant gene. The one F_1 plant and the two backcross plants without conversion could have arisen from pollen contamination during crossing.

The rapidity of the paper chromatography procedure also permitted an extensive study of the alkaloids of tobacco varieties. In a preliminary study of 36 individual plants of Ky 16 burley, nine plants were found to carry the conversion factor, and heterozygosity with respect to the nicotine level was indicated. An additional study was conducted, using 20 plants from each of 100 single plant lines of Ky 16. Homozygous and heterozygous lines for conversion and the extent of conversion were found. Some of these averaged 25 to 54 percent less nicotine than the average of the 100 lines. Conversion has also been found in other burley tobacco and in flue-cured varieties, and a widespread distribution of the conversion factor in *Nicotiana tabacum* is indicated.

Table 1. Data obtained from plants of the F_1, F_2, and BC_1 generations and the expected numbers assuming a single dominant gene *C* to control the conversion of nicotine to nornicotine.

Class of plants	Number of plants			
	Conversion (*CC or Cc*)		Nonconversion (*cc*)	
	Expected	Obtained	Expected	Obtained
F_1 *cc* × *CC*	36	35	0	1
F_2 *cc* × *CC*	120	114	40	46
BC_1 *Cc* × *cc*	30	32	30	28
BC_1 *Cc* × *CC*	50	48	0	2

Considering the heterozygosity of burley tobacco with respect to alkaloids, it seems probable that varieties will be heterozygous with respect to other chemical constituents. If this proves to be true, there is an opportunity to improve present varieties with respect to aroma, taste, and general smoking qualities by selection.

References and Notes

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