duly large. The solvent is allowed to rise to a prescored pencil line, 10 in. from the bottom edge of the filter paper. This takes 1½ hr. The blank produces a yellow spot on the finish line, and estrogens give in addition a purple[3] spot.

Table 1 gives the average R_f values of spots obtained by chromatographing estrone, estradiol, and estriol, singly and in mixture. As expected from the theory of partition chromatography, the R_f values of the three compounds decrease with increasing number of hydroxyl groups. Mixtures are readily resolved and identified. Only steroids containing a phenolic group give purple spots; this includes the equine estrogens. Diethylstilbestrol does not give the test. Double spots indicate a chemical change during the test, e.g., hydrolysis, or an impurity in the compound tested. Certain 3-ketosteroids, having a double bond in ring A, give yellow spots.

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[3] Absorption maximum around 540 mμ.

Enzymatic Activities of Isolated Normal and Mutant Mitochondria and Plastids of Higher Plants

H. G. duBuy, M. W. Woods, and Mary D. Lackey

Laboratory of Physical Biology,
Experimental Biology and Medicine Institute, and
National Cancer Institute,
National Institutes of Health, Bethesda, Maryland

The chloroplasts of higher plants derive from the mitochondrial elements of the cell (*4*, *6*), and possess extranuclear hereditary entities (chondriogenes [*1*]). Although it has been shown that isolated chloroplasts carry oxidizing enzymes (*7*), and that the activity of these enzymes may be deranged following chondriogene mutation (*9*), we are not aware of any reports dealing with the enzymic properties of the undifferentiated mitochondria in comparison with the chloroplasts. Furthermore, it is important to know whether plant plastids and mitochondria carry all or the major part of certain respiratory enzymes, as do animal mitochondria (*3*, *5*). It has been demonstrated that certain animal neoplasms are characterized by peculiarities in mitochondrial enzyme systems (*2*). Besides providing more information on extent of homology between plant and animal mitochondria, such data should also be of value in determining the role of mutant mitochondria in cell pathology, particularly in relation to disturbed metabolism and growth.

The genetically controlled plant material consisted of freshly harvested leaves of *Nicotiana tabacum* L. and *Lonicera japonica* Thunb.[1] The variegated (plastid mutant) *Nicotiana* material was secured by crossing an emasculated plant carrying the desired line of mutant mitochondria with a normal male parent. The F_1 plants were of the Maryland Medium Broadleaf type and, by virtue of somatic segregation of mitochondria, the leaves of the same plant often contained mesophyll cells with either normal, mutant, or normal and mutant mitochondrial elements. These mutant mitochondria are sexually inherited in non-Mendelian fashion through the female gametes and develop into chlorophyll-deficient plastids with cytological characteristics similar to type IIc variegations (*8*). The enzymic activity of centrifugally purified plastids and mitochondria of this material was compared with similar elements of normal cells.

Extractions were carried out at 5° C by grinding fresh leaf tissues in a Waring Blendor, in approximately four parts by weight of unbuffered sucrose solution (0.15 to 0.5 molar), and filtering through glass wool on a Buchner funnel. The filtrate (a cell-free homogenate) was compared with respect to its activity with fractions of two aliquots, fractionated by differential centrifugation in a Servall Superspeed angle-head centrifuge. One aliquot, subjected to 19,000 *G* for 20 min, yielded a "total pellet" and a supernatant devoid of microscopically visible particulates. The other aliquot, subjected to fractional centrifugations in order to remove nuclei, etc., yielded on sedimentation at 120 *G* for 10 min a pellet composed almost entirely of plastids, or plastids and starch. The plastids were sometimes subjected to additional cycles of suspension and sedimentation in fresh sucrose solution. The mitochondrial fraction was obtained by centrifugation of the supernatant at 19,000 *G* for 20 min following removal of the plastids. The mitochondria formed a compact brownish-gray pellet. Microscopically, they appeared mainly as colorless granules varying from 0.3 to 1 μ in diam. Some plastid fragments were always present in this fraction.

Considerable variability in enzymic activity, determined by Warburg technique, was encountered in leaves from plants of different ages or growth conditions. This variability was diminished by making comparisons between normal and mutant tissues derived either from equal areas of mutant and normal tissue of each leaf used, or from different leaves of approximately the same physiological age. Table 1 presents the results of some representative experiments in which composite tissue samples of normal and variegated leaves of approximately the same age were compared. Since preliminary data showed that the tissue extracts decreased auto-oxidation of the substrates, corrections were not made for auto-oxidation blanks. It was also determined that addition of cytochrome C to PPDA (paraphenylene diamine) did not stimulate oxidation in the absence of tissue fractions.

There is little difference between normal and mutant fractions with respect to the oxidation of PPDA with and without cytochrome C. Pronounced inhibition of oxygen uptake following addition of azide occurred mainly in the fractions containing particulates, indicating that the particulates are the main carriers of the cytochrome system. As would be expected, there was some decrease

[1] Acknowledgment is due to the Department of Botany of the University of Maryland for furnishing greenhouse facilities.

TABLE 1

ENZYMIC ACTIVITIES OF THE HOMOGENATE, TOTAL PELLET, AND SUPERNATANT FROM EQUAL VOLUMES (APPROXIMATELY EQUAL NUMBERS OF CELLS) OF NORMAL AND MUTANT (CONTAINING MUTANT MITOCHONDRIAL ELEMENTS) TISSUES OF *Nicotiana tabacum**

	Homogenate		Pellet		Supernatant	
A.† *PPDA*‡	Normal	Mutant	Normal	Mutant	Normal	Mutant
1st 25 min	−24.0 −24.0	−36.0 −24.0	− 5.6 − 7.1	− 5.4 − 6.4	−20.0 −19.6	... −29.2
2nd 25 min	−22.0 −18.8(Az)	−26.0 − 6.0(Az)	− 4.5 − 3.4(Az)	− 4.8 − 3.4(Az)	−22.0 −19.6(Az)	... −30.4(Az)
PPDA + cytochrome C§						
1st 25 min	−34.4 −36.0	−31.0 −24.8	−16.1 −14.4	−15.4 −15.2	−22.8 −19.8	−24.0 −22.0
2nd 25 min	−26.0 −18.0(Az)	−29.0 −24.0(Az)	−13.8 − 2.9(Az)	−15.2 − 5.2(Az)	−20.6 −20.8(Az)	−22.8 −19.0(Az)
Catechol‖						
1st 30 min	−298.2	−75.8	−80.8	−12.5	−206.4	−74.8
DOPA¶						
1st 30 min	−170.6	−92.0	−62.4	−13.1	−231.2	−83.2
B.† *PPDA*‡						
1st 30 min	−23.8 −35.0	−21.4 −22.0	− 6.9 − 8.1	− 7.9 − 9.1	−16.0 −21.4	−16.0 −21.8
2nd 30 min	−16.4 −11.8(Az)	−19.4 −13.2(Az)	− 4.4 − 1.8(Az)	− 9.5 − 2.5(Az)	−14.0 −12.6(Az)	−18.0 ...
PPDA + cytochrome C§						
1st 30 min	−32.2 −31.4	−27.0 −31.6	−21.7 −19.9	−18.3 −21.4	−14.2 −20.0	− 9.6 −21.0
2nd 30 min	−26.6 −10.0(Az)	−25.0 −11.4(Az)	−13.8 − 3.6(Az)	−13.9 − 3.7(Az)	....−14.0(Az)	− 9.6 −11.4(Az)
Catechol‖						
1st 30 min	−129.0 ...	−18.8 −20.8	−50.5 −54.7	− 8.2 − 6.9	−77.8 −79.6	−15.0 −14.6
C.† *PPDA*‡						
1st 30 min	−17.2 −21.4	−21.4 −21.2	− 4.4 − 5.6	− 6.1 − 5.6	−18.6 −18.6	−19.8 −22.6
2nd 30 min	−17.2 −16.8(Az)	−16.2 −17.4(Az)	− 5.9 − 4.4(Az)	− 7.4 ..	−19.6 −16.4(Az)	−16.0 −18.2(Az)
PPDA + cytochrome C§						
1st 30 min	−24.8 −23.2	−27.6 −27.8	−13.6 −14.0	... − 9.8	−24.2 −19.8	−12.8 −16.6
2nd 30 min	−22.4 − 5.6(Az)	−23.4 −20.8(Az)	−13.2 − 4.1(Az)	... − 2.6(Az)	−24.4 −18.8(Az)	−12.8 − 9.6(Az)
Catechol‖						
1st 30 min	−72.8 −68.0	− 7.2 0	−33.6 −34.9	− 0.7 − 0.6	−26.4 −39.2	0 0

* Activities are expressed as μl O_2 consumed per hr per ml homogenate equivalent.

† A, B, and C represent experiments on three separate lots of material.

‡ Paraphenylene diamine (PPDA), 0.02 M. Each value represents a determination on one vessel. Azide (Az), NaN_3, 10^{-3} M, added after lapse of first period.

§ Cytochrome C (mammalian), 10^{-5} M.

‖ Catechol, 0.02 M.

¶ DL-Dopa (dihydroxyphenylalanine), 0.02 M.

in enzyme activity with time. The phenolase activity of extracts of normal tissues was found to be significantly higher than that of comparable mutant tissues. Semi-qualitative colorimetric determinations of phenolase activity in five separate pairs of leaf extracts (addition of catechol, or tyrosine with a trace of catechol) also showed that normal tissues were more active than those of corresponding mutant tissues.

Table 2 presents the range of enzyme activities of isolated plastids and mitochondria from normal and variegated tobacco leaves. In comparing the Qo_2 values of mutant particulates with those of the normal plastids and mitochondria it should be kept in mind that the dry weights per unit area of leaf are lower for variegated than for normal tissues, which fact explains the higher values found for variegated tissues. Nevertheless, the values for phenolase activity for the mutant particulates are much lower than for normal particulates.

A series of comparable experiments were performed with fractions of normal *Lonicera* leaves, yielding data similar to those obtained with *Nicotiana*. Whereas *Nicotiana* fractions did not utilize lactate appreciably above the endogenous rate, *Lonicera* particulates did. Thus in a typical experiment a *Lonicera* homogenate gave a Qo_2 of 0.5 with cytochrome C 10^{-5} M, but without added substrate, and 3.6 with 0.02 M lactate plus cytochrome C. The corresponding values for plastids were 0.1 and 1.3, and for mitochondria 0.8 and 6.7. The supernatant fractions gave no gas uptake with lactate. The gas uptake with lactate as substrate was not decreased by the addition of .001 M azide.

From the data obtained, the following conclusions can

be drawn: (a) The particulate-containing fractions were the only ones in which the PPDA oxidation was markedly stimulated by the addition of cytochrome C in both mutant and normal tissues. (b) The same fractions were the only ones markedly inhibited after addition of azide. (c) Catechol was oxidized by normal tissue fractions at a much higher rate than by the fractions from mutant tissues. (d) The supernatant fraction showed considerable oxygen uptake in the presence of PPDA. This, however, did not involve the cytochrome system, as the addition of neither cytochrome C nor azide had any marked effect on the rate of oxidation.

TABLE 2

RANGE IN ENZYMIC ACTIVITIES OF ISOLATED PLASTIDS AND MITOCHONDRIA FROM NORMAL AND VARIEGATED LEAVES OF *Nicotiana tabacum**

Plastids	PPDA	PPDA + cyto-chrome C	Catechol	Endo-genous
Normal plastids	0.1	2.7	14.1	.5
	2.1	4.1	29.5	.2
	4.0	10.0	...	1.0
Normal mitochondria	2.0	3.7	13.7	.4
	2.6	5.9	18.1	.2
Mutant plastids	2.6	6.8	3.7	0
	1.8	11.2	0	.3
	2.7	11.9	...	.3
Mutant mitochondria	2.4	7.5	3.1	0
	4.1	21.6	0	1.4

* Each horizontal row represents data from one experiment. All activities are expressed as μl O_2 consumed per mg dry wt per hr and are based on duplicate determinations.

In general, the results obtained with *Nicotiana* and *Lonicera* show that both mitochondria and plastids carry all or the bulk of the cytochrome oxidase activity of the cell, thus providing further evidence for the homologous nature of plant and animal mitochondria. Furthermore, the data from *Nicotiana* demonstrate that mitochondrial mutation can result in marked derangement of certain enzyme systems of the cell, and that such abnormalities are apparent in both mitochondria and plastids.

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The Significance of Nonclassical Statistics

R. V. L. Hartley

Bell Telephone Laboratories, Murray Hill, New Jersey

Certain statements by Wiener (*3*) have led to the considerations given here, regarding the conditions under which the Gibbs type of statistics are and are not convenient for describing Newtonian systems. A parallel is traced between this situation and that which is met in wave mechanics, and the conclusion is reached that the success of nonclassical statistics in wave mechanics is not in itself evidence that atomic systems are not Newtonian.

The first statement, p. 110, is:

> In the Newtonian physics, the sequence of physical phenomena is completely determined by its past, and in particular, by the determinations of all positions and momenta at any one moment. In the complete Gibbsian theory it is still true that with a perfect determination of the multiple time series of the whole universe, the knowledge of all positions and momenta at any one moment would determine the entire future. It is only because these are ignored, nonobserved coordinates and momenta that the time series with which we actually work take on the sort of mixing property with which we have become familiar in this chapter, in the case of time series derived from the Brownian motion.

Then at the bottom of p. 117:

> . . . a dynamical system with no input may go into permanent oscillation, or even oscillation building up to infinity, with an undetermined amplitude. In such a case the future of the system is not determined by the past, . . .

Such a system obviously does not conform to the definition of a Newtonian system given in the first quotation. Since Wiener is presumably referring to macroscopic systems, the individual parts of which obey Newtonian laws, his statement implies that a non-Newtonian system may be constructed from Newtonian parts. We are therefore led to question the existence of a system such as he describes.

Certainly some systems which are capable of sustained oscillations do not go into that condition "with no input." A pendulum clock or a gasoline engine requires a starting input comparable in magnitude with the resulting oscillations. Certain types of vacuum tube oscillator, which depend on exact relations among the harmonics, will not oscillate for certain circuit adjustments unless they are subject to a fairly large input disturbance. By suitable design the magnitude of the required input may be reduced to the point where it is practically impossible to bring the system into a condition suitable for oscillations without having them start. Here the starting input may be of either of two forms. It may be a by-product of the adjustment which sets up the oscillatory condition. Here it is obviously determined by the past and nothing non-Newtonian is involved. If we assume that the system can be brought into a condition of unstable equilibrium and left there, then it will remain in that state indefinitely unless it is disturbed by some input. Because