tion of cytochrome reduction by substrate in the stripped membranes leads to the general conclusion that no essential component has been removed to the extent that electron transfer has been inhibited. The cytochrome assays rule out the presence of a_3, *a*, and *c* in the subunits, and the electron-transfer activity rules out DPNH and succinate dehydrogenases. However, our experimental method does not rule out oxidative phosphorylation cofactors or citric acid cycle dehydrogenases. The loss of some cytochromes *b* and c_1 from the stripped membrane is suggestive and needs further study. Thus, these experimental data appear to confirm our view (*4*, *5*) that the function of electron transport cannot be assigned to a single inner membrane subunit and confirms the possibility that the oxysome function of electron transport and oxidative phosphorylation involves a larger portion of the cristum than a single subunit and may extend along the cristum or into the cristum to meet the size requirements for the components of the oxysome (at least 170Å in diameter) (*5*). The possibility that other structural details may be identified with the oxysome function is suggested by the "dumbbell-like" structures of the cristum in flight muscle mitochondria (*9*).

Concerning the structure of the electron transport and oxidative phosphorylation components in the cristum, we find that the following hypotheses stand the test of continued experimentation. The respiratory chain consists of an assembly of cytochromes of approximately one each of the cytochrome (*10*) (particularly a_3, *a*, *b*, and c_1) with larger amounts of flavin NAD, quinone, and metal atoms; the respiratory components are intimately associated with the structure of the cristum, according to our early suggestions and those of Estabrook and Holowinsky (*11*) and Lehninger (*12*). Indeed, the idea of enzyme assemblies (*10*) has been extended to include a larger variety of enzymes of the cell, both in the citric acid cycle and in glycolysis under the term, "constant proportion enzymes" (*13*).

B. CHANCE
Johnson Research Foundation, University of Pennsylvania, Philadelphia

D. F. PARSONS
G. R. WILLIAMS
Departments of Medical Biophysics and Biochemistry, University of Toronto, Toronto, Canada

10 JANUARY 1964

References and Notes

1. D. F. Parsons, *Science* **140**, 985 (1963).
2. H. Fernandez-Moran, *Circulation* **26**, 1039 (1962).
3. D. Green, *Discovery*, Sept. 1962, p. 28.
4. D. Green, P. V. Blair, T. Oda, *Science* **140**, 382 (1963).
5. B. Chance, C. P. Lee, R. W. Estabrook, *Science* **140**, 379 (1963).
6. B. Chance, and D. F. Parsons, in preparation.
7. B. Chance and G. R. Williams, *J. Biol. Chem.* **217**, 383 (1955), I.
8. R. W. Estabrook in *Haematin Enzymes* (Symposium, Canberra, 1959), J. E. Falk, R. Lemberg, R. K. Morton, Eds. (Pergamon, Oxford, 1961), p. 436; B. Chance, *ibid.*, p. 597; W. D. Bonner, Jr., *ibid.*, p. 479.
9. D. S. Smith, *J. Cell Biol.* **19**, 115 (1963).
10. B. Chance, *Nature* **169**, 215 (1952); B. Chance and G. R. Williams, *Advan. Enzymol.* **17**, 65 (1956).
11. R. W. Estabrook and A. Holowinsky, *J. Biophys. Biochem. Cytol.* **9**, 19 (1961).
12. A. L. Lehninger, *Sci. Am.* **9**, 62 (1961).
13. D. Pette, M. Klingenberg, T. Bücher, *Biochem. Biophys. Res. Commun.* **7**, 425 (1962); M. Klingenberg and D. Pette, *Biochem. Biophys. Res. Commun.* **7**, 430 (1962).
14. We thank Brigitte Schoener, Reiko Oshino, and Tamiko Yoshioka for technical assistance. Supported in part by grants from the National Science Foundation and the U.S. Public Health Service.

29 October 1963

Diploid and Endoreduplicated Cells: Measurements of DNA

Abstract. *Two-wavelength microspectrophotometry has shown that endoreduplicated cells in an x-irradiated culture of human leukocytes contained about twice as much DNA as diploid cells from the same culture. This supports the assumption that endoreduplicated cells represent a form of polyploidy.*

The doubling of the number of visible chromosome elements, resulting in four-stranded chromosomes, is termed endoreduplication. This phenomenon has been induced in cultures of human leukocytes by various forms of ionizing radiation (*1*). It has been generally assumed that endoreduplication represents a form of polyploidy, that is, that the chromosome set and the DNA content are doubled. It was considered important that this assumption be verified, since the possibility has been entertained that endoreduplication is due to subdivision of multistranded chromatids. Microspectrophotometric measurements were therefore made of the DNA content of normal diploid and of endoreduplicated cells at metaphase in an irradiated culture of human leukocytes.

The leukocyte culture was prepared by modifications of the techniques of Moorhead *et al.* (*2*). Endoreduplication was induced by x-irradiation. Radiation was supplied by a Philips x-ray machine, operated at 15 ma and 250 kv. Filtration was provided by 1-mm Al and 0.25-mm Cu. Dose was measured with a Victoreen condenser roentgen meter and 250-r chamber. The culture was exposed to 400 r of x-irradiation at 202 r/min, 48 hours after it had been prepared. The cells were harvested after another 48 hours, and slides were prepared, which, after drying in air, were stained by the Feulgen procedure, mounted in oil (refractive index 1.545), and sealed with paraffin.

To reduce to a minimum errors due to variations in staining, only one slide was used. This was the slide judged to have the greatest number of suitable metaphase figures. For microspectrophotometric measurements, cells had to be selected which were well separated from other cells. In addition, the chromosomes had to be well bunched together, but not so much that identification of diploidy and endoreduplication would be difficult. This made accurate chromosome counts impossible, but since the cells were apparently intact it was assumed that no loss of DNA material had occurred.

Nine diploid and five endoreduplicated cells fulfilled these criteria. Measurements of DNA were made by the two-wavelength method (*3*) by means of a Canalco microspectrophotometer. The data obtained are presented in Table 1.

From these results it is apparent that the endoreduplicated cells contain more DNA than do diploids, and that their presence, therefore, cannot be due to simple chromatid splitting. Although the confidence limits are fairly wide, the endoreduplicated cells appear to have about twice the DNA content as the diploids. This is consistent with the aforementioned assumption about en-

Table 1. Microspectrophotometric measurements of DNA in diploid and in endoreduplicated cells at metaphase.

Diploid	Endoreduplicated
400	682
430	601
422	797
411	982
431	1081
538	
365	
497	
393	
Mean	
431 ± 18	829 ± 90
95 percent confidence limits	
389–473	597–1079

doreduplication, and with the hypothesis that a complete second round of DNA synthesis has occurred in one mitotic cycle of these cells.

ALEX. G. BELL*
Biology Division, Oak Ridge National Laboratory,† Oak Ridge, Tennessee

References and Notes

1. Y. Ohnuki, A. Awa, C. M. Pomerat, *Ann. N.Y. Acad. Sci.* **95**, 882 (1961); A. G. Bell and D. G. Baker, *Can. J. Genet. Cytol.* **4**, 340 (1962); A. G. Bell and D. G. Baker, in preparation; P. C. Gooch and M. A. Bender, personal communication.
2. P. S. Moorhead, P. C. Nowell, W. J. Mellman, D. M. Battips, D. A. Hungerford, *Exptl. Cell Res.* **20**, 613 (1960).
3. K. Patau, *Chromosoma* **5**, 341 (1952).

* Present address: Department of Human Genetics, University of Michigan, Ann Arbor.

† Operated by Union Carbide Corporation for the U.S. Atomic Energy Commission.

7 October 1963

Glucose-6-Phosphate Dehydrogenase in Drosophila: X-Linked Electrophoretic Variants

Abstract. *Three electrophoretic patterns of glucose-6-phosphate dehydrogenase have been found in wild-type strains of* Drosophila melanogaster. *Genetical control of the variants in enzyme mobility (fast or slow) is X-linked; strains possessing both bands (fast and slow) appear to be heterogeneous, having individuals of three types (fast, slow, and double).*

Although an enormous number and variety of mutant phenotypes is known in *Drosophila melanogaster*, there are in this organism only six well-characterized gene-enzyme systems available for analyzing the genetical control of protein structure (*1*). In man, on the other hand, where genetic analysis is much more tedious, there is a broad range of biochemical properties whose variants are under genetical control. The absence of glucose-6-phosphate dehydrogenase in the erythrocytes of primaquine-sensitive human beings is inherited as an X-linked trait (*2*). The qualitative variants of this enzyme, discovered by starch-gel electrophoresis, also segregate with the X chromosome (*3*). It seemed reasonable to suppose that qualitative polymorphism is present and identifiable in *Drosophila*, as well as in human, populations.

A search for such a polymorphism was undertaken by examination of homogenates of 50 to 100 flies, and of single flies of various wild-type strains, by vertical starch-gel electrophoresis. The procedure was modified from those previously reported (*3*).

The flies were ground in 0.5 ml of NADP (*4*) (2mg/ml), and the homogenates were cleared by centrifugation at 0°C. Samples of the supernatants were loaded into ten-slot vertical gels (*5*) and 5 to 6 volts/cm were applied for 12 to 16 hours at 4°C. The gel (Connaught starch, hydrolyzed) was prepared in 0.05*M* tris HCl (*6*) at *p*H 8.6, and the bridge solution was 0.05*M* tris at the same *p*H. Five milliliters of 0.27*M* EDTA and 4 mg of NADP were added to the starch before pouring. Sliced gels were developed in the dark at 25°C for 2 to 12 hours in a solution containing 80 ml of 0.5*M* tris HCl, *p*H 8.6; 10 ml of 0.01*M* $MgCl_2$; 50 mg glucose-6-phosphate; 10 to 20 mg NBT (*7*); 8 mg phenazine methosulfate, and 10 mg NADP. Single flies were ground in microhomogenizers (*8*) and the uncentrifuged homogenates were loaded directly onto the gel.

Three different electrophoretic patterns of the glucose-6-phosphate dehydrogenase occur among various wild-type strains of *D. melanogaster*. We found, using mass homogenates, that of the 19 wild-type stocks from various localities, five show only a single slow-moving band; twelve have only a single fast-moving band; and two strains present both fast and slow bands. The appearance of these bands is illustrated in Fig. 1.

Although the photograph is of a gel in which there were only homogenates of single flies, the patterns are typical of the multiple-fly homogenates. Numbers 2, 3, and 6, are bands of fast-moving dehydrogenase, 4 of slow, and 1, 5, and 7, of double. In the homogenates of flies illustrated here, the leading component of the double band is more intensely stained; in other strains, the slower component stains more brilliantly.

Table 1. Distribution of glucose-6-phosphate dehydrogenase phenotypes in offspring in *D. melanogaster* as a result of "fast × slow" (electrophoretic mobility of dehydrogenase) mating. The numbers in parentheses indicate numbers of individuals examined.

Parents		Offspring	
Male	Female	Male	Female
Fast	Slow	Slow (13)	Double (13)
Slow	Fast	Fast (11)	Double (12)

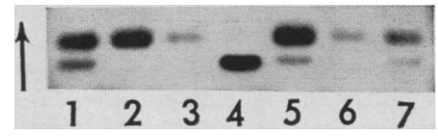

Fig. 1. Glucose-6-phosphate dehydrogenase phenotypes of single flies from mating of slow male (No. 4) by fast female (No. 3). The F_1 males are Nos. 2 and 6; F_1 females are Nos. 1, 5, and 7. The arrow indicates direction of migration.

These observations clearly establish polymorphism for this dehydrogenase in *Drosophila* populations. Since the strains containing the fast and slow enzyme variants each appear to be phenotypically constant, and the "doubles" are apparently heterogeneous, no information on the mode of inheritance is provided, except the inference of a pair of codominant alleles. Examination of single flies from one strain (Woodbury, New Jersey) suggests X linkage as a probable mode of inheritance, since we have observed three phenotypes in females (10 slow, 11 double, 48 fast), and only two phenotypes in males (2 slow, 25 fast).

Two additional lines of evidence, however, establish the X-linked control of this polymorphism:

1) Various combinations of single pair matings were made. In matings of flies containing slow bands, all progeny had slow bands; these matings are uninformative. However, the fast × slow matings provided critical information on the mode of inheritance. The phenotypes of the parents and offspring of such pair matings are given in Table 1 and are illustrated in Fig. 1. The results of the reciprocal matings are entirely consistent with transmission by way of the X chromosome. That is, in both cases all F_1 males have the maternal phenotype, and the F_1 females, whose X chromosomes come from both parents, are invariably double-banded.

2) Single flies from two stocks of attached-X females were examined. In as much as the males in these stocks are patroclinous, then if the character in question is X-linked all males from a given stock should be identical. If the phenotype of the females happens to differ, then the two phenotypes will segregate strictly according to sex. Seventy-four individual flies were examined; all of the 38 males were classed as *slow*; all of the 36 females were classed as *fast*. The only chromosome possessed by all the males but by none