1 Acknowledgments. This work was supported by a grant from Consejo Nacional de Investigaciones Científicas y Técnicas República Argentina (EFIHER - CONICET). Isolated rat liver preparations were performed by Miss Graciela Patricia Rodriguez. The assistance of Carlos Guillermo Grieve is also gratefully acknowledged.
2 Author for correspondence.
3 J.M. Céspedes and E.A. Rodriguez Garay, Acta physiol. latinoam. *29*, 207 (1979).
4 M. Frezza, C. Tiribelli, E. Panfili and G. Sandri, FEBS Lett. *38*, 125 (1974).
5 C. Tiribelli, G.C. Lunazzi, M. Luciani, E. Panfili, B. Gazzin, G.F. Liut, G. Sandri and G.L. Sottocasa, Biochim. biophys. Acta *532*, 105 (1978).
6 G. Litwack, B. Ketterer and I.M. Arias, Nature *234*, 466 (1971).
7 B. Combes and G.S. Stakelum, J. clin. Invest. *39*, 1214 (1960).
8 G. Whelan, J. Hoch and B. Combes, J. Lab. clin. Med. *75*, 542 (1970).
9 C.D. Klaassen, J. Pharmac. exp. Ther. *168*, 218 (1969).
10 B.G. Priestly and G.L. Plaa, Proc. Soc. exp. Biol. Med. *135*, 373 (1970).
11 C.D. Klaassen, Biochem. Pharmac. *19*, 1241 (1970).
12 C.D. Klaassen, J. Pharmac. exp. Ther. *195*, 311 (1975).
13 B. Solymoss and G. Zsigmond, Can. J. Physiol. Pharmac. *51*, 319 (1973).
14 A.D. Mottino and E.A. Rodriguez Garay, Biochem. Pharmac. *28*, 2215 (1979).
15 G. Zsigmond and B. Solymoss, Proc. Soc. exp. Biol. Med. *145*, 631 (1974).
16 G. Zsigmond and B. Solymoss, J. Pharmac. exp. Ther. *183*, 499 (1972).
17 C.D. Klaassen, Can. J. Physiol. Pharmac. *53*, 120 (1975).
18 D.G. Shand, D.M. Kornhauser and G.R. Wilkinson, J. Pharmac. exp. Ther. *195*, 424 (1975).
19 F.L. Rodkey, Clin. Chem. *2*, 478 (1965).
20 R.W. Brauer, R.L. Pessotti and P. Pizzolato, Proc. Soc. exp. Biol. Med. *78*, 174 (1951).
21 B. Kotelański, R.J. Groszmann, J. Kendler and H.J. Zimmerman, Proc. Soc. exp. Biol. Med. *132*, 715 (1969).
22 B.A. Noir, E.A. Rodriguez Garay and M. Royer, Biochim. biophys. Acta *100*, 403 (1965).
23 N.B. Javitt, H.O. Wheeler, K.J. Baker, O.L. Ramos and S.E. Bradley, J. clin. Invest. *39*, 1570 (1960).
24 E.A. Rodriguez Garay, V. Pérez and M. Royer, Acta physiol. latinoam. *17*, 68 (1967).
25 F. Afifi, M. Peignoux and C. Anclair, Hepato-Gastroenterol. *27*, 9 (1980).
26 H. Reyes, J.A. Levi, Z. Gatmaitan and I.M. Arias, J. clin. Invest. *50*, 2242 (1971).

Influence of oxygen concentration on development of *Drosophila melanogaster*[1]

H. Nickla, D. Fujiwara and R. Fried

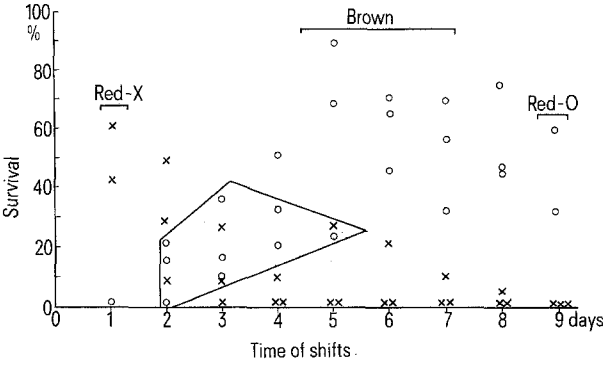
Departments of Biology and Biochemistry, Creighton University, Omaha (Nebraska 68178, USA), 1 June 1981

Summary. 1st, 2nd, and early 3rd instar *Drosophila* larvae are extremely sensitive to 100% O_2 or 75% O_2/25% N_2 (at atmospheric pressure) whereas eggs, late 3rd instar larvae, and pupae are relatively insensitive under our exposure conditions. Eclosing flies exposed to an O_2 enriched environment consistently possessed 2 eye abnormalities: dark eye color and altered eye shape.

Approximately 1.3 billion years ago[2] photosynthesis provided sufficient free oxygen (1% of its present value) for utilization of a superior strategy (aerobic metabolism) for the production of high energy phosphate bonds. Compared to anaerobic processes, metabolism with free O_2 confers a 20:1 efficiency advantage. However, all taxa appear to be sensitive to oxygen poisoning[3], and it is generally accepted that oxygen is potentially toxic at all concentrations[4]. For aerobes, mechanisms have evolved which protect tissue from oxygen-generated free radical damage at normal (air) concentrations. These mechanisms include catalase, glutathione peroxidase, superoxide dismutases, and DNA repair mechanisms[2,4]. Current literature supports a model which suggests that the inability of these mechanisms to manage oxygen-generated superoxide and hydroxyl radicals and hydrogen peroxide, causes the accumulation of undesirable or non-functional cellular substances associated with aging tissue[5,6].

The sensitivity of adult *Drosophila* to poisoning by oxygen has been extensively documented[3,7-9]. While the effects of short-term elevated O_2 exposure are largely reversible, long-term exposures apparently damage the nervous system[3,9]. In this paper we describe the influence of increased atmospheric oxygen (at normal atmospheric pressure) on development of 2 *Drosophila* strains, *vestigial wings*[10] (at position 67.0 on chromosome 2) and wild type (Urbana). The vestigial strain was selected because of its shortened life span[11] and reported decrease of superoxide dismutase activity in adults when compared to wild type[12].

Eggs were collected at 8 h intervals and placed 50-each in shell vials containing 5 cm^3 of standard cornmeal, molasses, sucrose, agar medium, Each vial was stoppered with a single layer of coarse-mesh nylon. Such vials were initially placed in a lucite chamber containing either a 20% O_2/80% N_2 gas mixture or a similar chamber containing 100% O_2. At 24-h intervals 1 (or 2) vial from each chamber was reciprocally shifted[13]. After 14 days from the onset of each experiment the percentage of surviving adults was determined. 3 replications of this procedure using a total of 5850 eggs provided the points (represented by symbols O = 20% O_2/80% N_2 → 100% O_2; X = 100% O_2 → 20% O_2/80% N_2) presented in the figure. A zone of overlap exists in days 2 through 5 thereby indicating that larvae are highly sensitive to 100% O_2 at 1st, 2nd, and early 3rd instars. Eggs, late 3rd-



Percentage survival (adult flies) of developing vestigial *D. melanogaster* exposed to 100% O_2 and transferred to a 20% O_2/80% N_2 gas mixture (X) and the reverse (O) at 24-h intervals at room temperature (24 ± 1 °C). See table for explanation of Brown, Red-X and Red-O terms. Symbols in the same column represent results from 3 replicate experiments conducted on a total of 5850 eggs.

Fluorescent pigments in eyes, testes, and abdomens of adult *D. melanogaster* (aged 2–3 days) exposed to 100% O_2 at the egg stage (Red-X in the figure), early to midpupal stage (Brown in the figure) and late pupal stage (Red-O in figure)

Compounds	Strain Vestigial Red-X	Brown	Red-O	Urbana Red-X	Brown	Red-O	F-Ratio	95% Confidence Limits ±	Tissue	Sex
Drosopterins	57.0	43.7	54.7	51.5	39.8	55.7	16.14**	4.42	Eyes	F
HB	27.0	70.5	28.5	31.5	78.2	35.0	148.11**	3.88	Eyes	F
Sepiapterin	57.7	57.8	52.8	49.2	55.0	47.5	3.62	4.68	Eyes	F
Xanthopterin-isoxanthopterin	60.5	56.5	55.3	63.3	72.5	60.7	3.87	6.43	Eyes	F
Sepiapterin				26.5	14.2	20.2	°°9.60*	4.82	Testes	M
Isoxanthopterin				76.8	43.0	51.5	20.57*	9.44	Testes	M
Yellow pigment	(primarily riboflavin)			64.2	31.5	75.8	23.31*	11.60	Abdomens	F
Isoxanthopterin				29.2	31.8	24.8	1.46	7.17	Abdomens	F

Values given are means (n = 6 for each) based on an arbitrary scale from 0 to 100. Because the fluorometer is reset when different compounds are measured, comparisons may not be made among means of different compounds. * $p < 0.05$; ** $p < 0.01$. HB = 2-amino-4-hydroxypteridine and biopterin (measured together).

instar larvae, and pupae show no appreciable loss of viability when exposed to 100% O_2. Male and female larvae appear to be equally sensitive. The results presented in the figure are for the vestigial strain. Replicate experiments with the wild strain yielded a similar O_2 sensitive period. Considerable variation exists in response (survivorship) to 100% O_2 under our exposure conditions. The source of this variation is yet to be determined; however, different larval feeding behaviors may create a variety of microhabitats (food tunnels) which influence the level of exposure of each organism. Even though both the vestigial and wildtype strains are highly inbred, it is also possible that genetic heterogeneity (perhaps for the protective mechanisms mentioned above) may be a component of the differential response.

Adult flies which spent at least 24 h of their early pupal life (prepupa to midpupa - pupation to onsent of bristle pigmentation) had brown eyes in contrast to the normal dull red eye color of flies not exposed during this period. Samples of flies having brown eyes (designated in the figure) and those having normal eye color (Red-X and Red-O in the figure) were examined chromatographically and fluorometrically[14] for changes in pigmentation of selected anatomical sites[15] (table). Red-eyed flies (Red-X and Red-O) of both vestigial and Urbana strains had more drosopterins than those flies exposed to 100% O_2 as early pupae (brown-eyed flies). HB pigments consisting of 2-amino-4-hydroxypteridine and biopterin (measured together) are greatly increased in brown-eyed flies as compared to their red-eyed counterparts. Amounts of sepiapterin and xanthopterin and isoxanthopterin (measured together) are similar in brown-eyed and red-eyed flies. Ommochromes (brown eye pigments) were not assayed. Testes of brown-eyed flies had less sepiapterin than red-eyed flies exposed to 100% O_2 as eggs (Red-X), whereas the amount of isoxanthopterin testes of brown-eyed and red-eyed flies exposed to 100% O_2 in late pupation (Red-O) was decreased compared to red-eyed (Red-X) flies. Yellow pigment (primarily riboflavin[14]) in female abdomens is decreased in brown-eyed flies as compared to their red-eyed counterparts. Significant changes were not observed for abdominal isoxanthopterin in females.

In addition to the sensitivity of pigmentation to increased O_2 levels, certain cells of the eye imaginal disk appear to be quite sensitive to elevated O_2 throughout larval and pupal development. Although not observed among survivors of 100% O_2 exposure, approximately one-third of the eyes of flies exposed to 75% O_2/25% N_2 during any developmental stage exhibit abnormal eye shape. In such cases, ommatidia are missing in the anterior-ventral portion of the eye. The defect ranges from severe in which approximately one-third of the eye is missing, to mild in which only a small notch is observed.

The toxicity of elevated atmospheric O_2 has been well documented[4] in a variety of adult organisms including *Drosophila melanogaster*[3,8]. Under our exposure conditions we found that developing *Drosophila* exhibit an O_2 sensitive period in the larval stage. Although a comprehensive study of levels of O_2-protecting enzymes (mentioned above) has not been conducted in developing *Drosophila,* Armstrong and co-workers[16] reported that peroxidase activity is lower in *Drosophila* larvae as compared with pupae and young adults. In addition, eggs, larvae, and pupae of the Meditarranean fruit fly *(Ceratitis capitata)* have lower activities of superoxide dismutase than adult flies with the lowest activities found in eggs and larvae[17]. Such reduced enzyme activities could account for our observations of increased O_2 sensitivity in larvae. It is of interest however that some larvae within the same culture vial are considerably less sensitive to elevated O_2 levels than other larvae. The nature of this variability is presently under investigation as is the influence of O_2 on pigmentation and imaginal (eye) disk development.

1 Supported by NSF-URP grant SP1782684 and a Summer Faculty Fellowship to Harry Nickla.
2 D. Harman, Age *3,* 100 (1980).
3 C. Williams and H. Beecher, Am. J. Physiol. *140,* 566 (1944).
4 B. Halliwell, Cell Biol. int. Rep. *2,* 113 (1978).
5 D. Harman, Age *3,* 64 (1980).
6 J. Miguel, A. Tappel, C. Dillard, M. Herman and K. Bensch, J. Geront. *29,* 622 (1974).
7 W. Fenn, Respir. Physiol. *3,* 117 (1967).
8 E. Block, Aviat. Space envir. Med. *48,* 645 (1977).
9 G. Kloek, M. Woelfel, and T. Kelly, Aviat. Space envir. Med. *49,* 587 (1978).
10 D. Lindsley and E. Grell, Genetic Variations of Drosophila melanogaster. Carnegie Inst. Wash. Publ. 627, 1968.
11 B.M. Gonzalez, Am. Nat. *57,* 289 (1923).
12 G. Bartosz, W. Leyko, and R. Fried, Experientia *35,* 1193 (1979).
13 T. Grigliatti and D. Suzuki, Proc. natl Acad. Sci. *67,* 1101 (1970).
14 H. Nickla, Can. J. Genet. Cytol. *14,* 105 (1972).
15 E. Hadorn and H. Mitchell, Proc. natl Acad. Sci. *37,* 650 (1951).
16 D. Armstrong, R. Rinehart, L. Dixon and D. Reigh, Age *1,* 8 (1978).
17 J. Fernandez-Sousa and A. Michelson, Biochem. biophys. Res. Commun. *73,* 217 (1976).