Vol. 112, No. 2, 1983 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 29, 1983 Pages 602-605

ELECTRON SPIN RESONANCES OF A LIVING SYSTEM (DROSOPHILA) ON NORMAL AND CARCINOGENIC DIETS

Charles Trapp, Bradford Waters, Gary Lebendiger and Miriam Perkins

Department of Chemistry, The University of Louisville,
Louisville, Kentucky 40292, USA

Received March 14, 1983

SUMMARY: This investigation was a study of the free radical concentration of a living system, Drosophila melanogaster, by electron spin resonance spectroscopy. The paramagnetic content of the organism was measured as a function of age, mutant strain, and diet (normal vs. carcinogenic). In all cases, 4 to 6 days after ecdysis the free radical concentration decreased to approximately 70% of its value as measured shortly after ecdysis. The different mutant strains exhibited distinctly different free radical concentrations in accord with visual observations of the degree of pigmentation. Drosophila raised on a carcinogenic diet always showed a lower concentration of free radicals than the control groups on a normal diet.

All biological materials tested by electron spin resonance (ESR) have been shown to contain free radicals (1,2), though at rather low concentrations. Most previous ESR studies on normal and cancerous tissues have employed samples very different from the tissues of a whole living system (1,2). The specimens have most often been taken from mammals, and the small size of the ESR cavity dictated the methods of preparation. The excised tissues were either lyophilized, rapidly frozen, or chilled in order to avoid autolysis. The samples studied were far removed from an *in vivo* state, and the different methods of sample preparation resulted in considerably different ESR intensities (1,2).

For these reasons, and the fact that Drosophila have been used extensively in recent years for the evaluation of carcinogens and mutagens (3,4), we have chosen this species for our ESR studies. There have been no previous ESR studies of a whole living organism as a function of age, mutant strain, and diet (normal *vs.* carcinogenic). However, other previous ESR studies (1,2) have indicated that excised cancerous tissues exhibit a lower (10-40%) concentration of free radicals than excised normal tissues. In this study we were

0006-291X/83 $1.50

interested in the effect of a carcinogen in the diet irrespective of any induction of cancer.

METHODS AND PROCEDURES: The fruit flies were raised on a diet of Formula 4-24 Instant Drosophila Medium obtained from Carolina Biological Supply, Inc. In the case of carcinogenic diets, 1% by weight of the carcinogen was added to the medium. Three strains of Drosophila were employed: the ebony bodied mutant, wild type Drosophila, and the white-eyed yellow-bodied mutant. The carcinogens employed were 2-acetylaminofluorene, which has been shown (5) to cause a number of abnormalities, including tumors, in Drosophila, as well as, benzopyrene, benzidine, and dimethylaminoazobenzene, all standard carcinogens.

A Varian Associates V-4502-12 EPR spectrometer was employed for the measurements. ESR spectra were taken daily with 20 flies in each sample tube. A minimum of three spectra were taken on each sample on a given day. The same flies were used throughout the duration of the experiment by returning the flies to their medium after the spectra were taken. The entire experiment was repeated a minimum of three times for each variety of fly and for each type of diet. Relative free radical concentrations could be determined to better than ±5% as demonstrated by comparing measurements on similar but different samples. No attempt was made to determine absolute magnitudes of concentrations accurately, but a rough estimation based on standardization of the spectrometer with DPPH (diphenyl picrylhydrazyl) gave approximately 10^{-10} moles of free radical per fly, or about 10^{16} radicals per gram of fly for the wild type Drosophila.

RESULTS AND DISCUSSION: The results are shown in the figure. In all mutant strains the spectra of Drosophila revealed a single structureless line with g = 2.0040 and a width of 6×10^{-4} T (6 gauss). The lineshape was symmetrical and closely approximated a Lorentzian.

The results indicate that the free radical concentration varied sharply as a function of age. After 4-6 days the concentration fell to 70% or less of the initial value except in the white-eyed yellow-bodied mutant where the drop was less evident. This was the case irrespective of the diet. The mutant varieties showed a distinct difference in radical concentration. The wild type had a concentration of about 60% of the concentration of the ebony-bodied strain, and the white-eyed yellow-bodied strain had a concentration of about 25% of that of the ebony-bodied strain. In all cases the addition of a carcinogen to the diet decreased the free radical content of the flies. In no case was an increase observed. The decrease varied from about 5% (curve 5) to about 30% (curve 8) depending upon the strain and carcinogen employed.

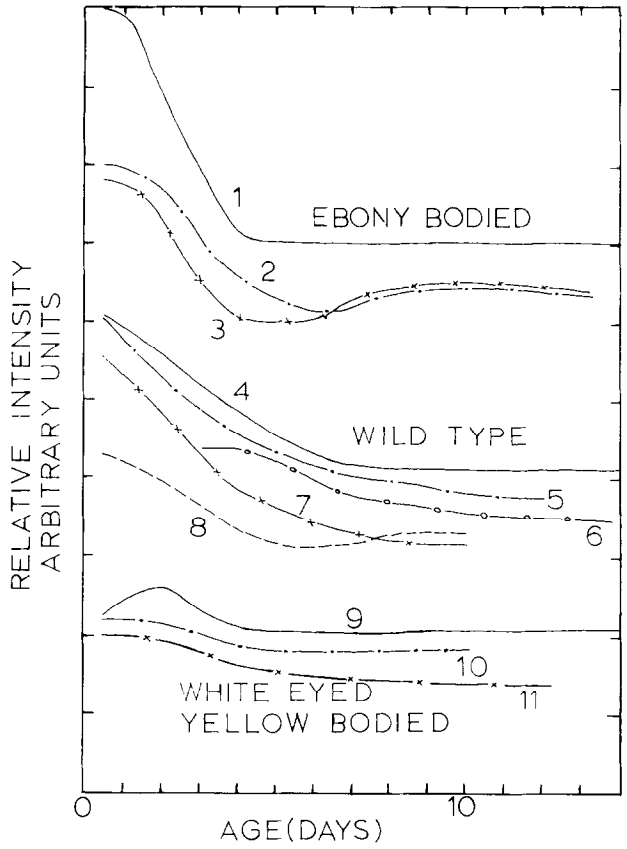

Figure 1. Relative free radical concentration as a function of age for three strains of Drosophila on normal and carcinogenic diets. Curves 1, 4, and 9: normal diet. Curves 2, 5, and 10: 1% benzo(a)pyrene. Curves 3, 7, and 11: 1% acetylaminofluorene. Curve 6: 1% benzidine. Curve 8: 1% dimethylaminoazobenzene. The markings on the curves are not data points. They are used for visual distinction between the curves. The scatter of data points is typically ±5% and are too numerous to be shown individually.

A major portion of the observed ESR signal intensity is undoubtedly due to free radicals in melanin, as is evident by comparing the results on the ebony-bodied and wild type to the results on the white-eyed yellow-bodied mutant. However, the change in intensity of the ESR signal with age in Drosophila also indicates variations in concentration of metabolic free radicals. After ecdysis the flies change color rapidly from almost white to their final coloration. This process is essentially complete after 6 hours, and thereafter changes in melanin concentration may be small. Unfortunately, it was not possible to obtain a sample of 20 flies all of which emerged within less than a 10 hour period. Thus the flies in any given sample had ages of about

15 hours, and is the reason that the curves in the figure do not extend to 0 days.

The observed drop in ESR signal intensity with age is not paralleled by any apparent decrease in color. The observed drop could be a reflection of lower metabolic free radical content as a result of a lower metabolic rate due to aging. Such a linkage has been proposed by Boenig (6). As the first few days after ecdysis present a high degree of physiological activity, it is not surprising that the maximum free radical concentration occurs within this interval.

The observed decrease in free radical concentration with Drosophila raised on a carcinogenic diet parallels the data observed on normal vs. cancerous mammalian tissues (1,2). Several explanations exist for this latter result (2,7) which will not be discussed here. We remark again, however, that we have observed this decrease in free radical concentration in a living system. The question remains as to whether the decreased ESR signal is due to a reduction of the concentration of metabolic free radicals, or of free radicals in melanin, or a combination of both. One might expect that free radicals in melanin would be rather inert to carcinogens in the diet, but this remains to be established. The fact that a somewhat larger percentage decrease in free radical concentration occurs in the white-eyed yellow-bodied mutant is evidence that the carcinogens influence the concentration of metabolic free radicals.

REFERENCES

1. D. C. Borg, (1976) in "Free Radicals in Biology," Academic Press, Inc., New York, Vol. I (1976); Chapter 3, 69-147.
2. P. F. Knowles, D. Marsh, and H. W. E. Rattle (1976) "Magnetic Resonance of Biomolecules," John Wiley and Sons, Ltd., London (1976); Chapter 7, 208-236; Chapter 8, 237-295.
3. E. Vogel, W. G. H. Blijleven, P. M. Klapwijk, and J. A. Zijlstra (1980), "Some Current Perspectives of the Application of Drosophila in the Evaluation of Carcinogens" in "The Predictive Value of Short-Term Screening Tests in Carcinogenicity Evaluation," G. M. Williams, et al., Eds., Elsevier/North-Holland Biomedical Press, 125-147.
4. A. J. Baars (1980), Drug Metabolism Reviews, 11, 191-221.
5. J. Schultz and N. Bischoff (1953), Am. Assoc. Cancer Res., 1, 48-52.
6. H. V. Boenig (1966), J. Am. Ger. Soc., 14, 253-258.
7. A. Szent-Györgyi (1977), Int. J. Quant. Chem., 12 (Suppl. 1), 407-414.