

glands is known to be rich in mucoproteins which are large molecules generally unable to enter an acrylamide gel of the concentration used here (7%). On the other hand, the glue also contains proteins of smaller size⁶. The *D. pallidipennis* protein band 86 as well as the equivalent *D. pseudoobscura* protein band (band 55) probably belong to this category.

The protein bands 50 and 58 which appear at the 160 h stage could be under ecdysone control. But while band 58 is specific of this stage and may have a function related to glue secretion, band 50 is also present at the 20 h after SE stage and might be related to molting (pupal molt at 160 h and pupal molt around 20 h after SE). Identical behaviors were observed for none of the *D. pseudoobscura* protein bands.

A very interesting observation is that new protein bands appear at the 20 h after SE stage, i.e. a few hours before the complete histolysis of the salivary glands. 2 protein bands were never present before this stage, i.e. bands 76 and 93. That 2 completely new proteins also appear at an identical developmental stage in *D. pseudoobscura* salivary glands^{3,7}, bands 82' and 85', is striking. In both cases these 2 bands were not detected in other tissues; therefore they are probably tissue specific. Further investigation is undoubtedly needed in order to determine whether these proteins are synthesized de novo at this stage or come from proteins of high molecular weight which have been partially degraded. This could be of particular importance for possible correlation with puffing patterns, the study of which has shown the formation of new puffs very late in the development of the salivary glands^{8,9}.

The study of the developmental pattern of unspecific proteins of the salivary glands of *Drosophila pallidipennis*

has thus confirmed several observations made with *Drosophila pseudoobscura*, i.e. the disappearance of certain protein components at the time of spiracle eversion and the appearance of new such components a few hours before the complete histolysis of the salivary glands. These observations suggest that such phenomena may take place throughout the genus *Drosophila*¹⁰.

Résumé. Les protéines présentes dans les glandes salivaires de *Drosophila pallidipennis* ont été analysées grâce à une technique de microélectrophorèse discale. Les variations observées pendant le développement de la larve et de la jeune pupa ont été comparées avec celles de *Drosophila pseudoobscura*. Il semble que certaines variations soient le résultat de phénomènes communs à toutes les espèces du genre *Drosophila*.

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⁸ A. J. STOCKER and C. D. KASTRITSIS, Chromosoma, in press.

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¹⁰ Acknowledgments. I am grateful to Dr. C. D. KASTRITSIS for reading the manuscript and for his suggestions.

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Constant Oxygen Enhancement Ratio During the Mitotic Cell Cycle in X-rayed *Drosophila melanogaster* Embryos

With a variety of cell types, a strong stage-dependent variation of the radiosensitivity during mitosis or meiosis is observed. This was demonstrated by WÜRGLER for the mitotic stages of the early cleavage divisions in *Drosophila melanogaster* (WÜRGLER, ULRICH and SCHNEIDER-MINDER¹). He used an egg collection technique (WÜRGLER, ULRICH and SPRING²) which allows the obtaining of samples with ample numbers of eggs within 3 min. These samples were exposed to X-rays at various times after collection. For convenience, the cells in samples, which are older than 15 min and which in fact are early embryos, are still called 'eggs'.

A cyclic variation of the radiosensitivity with age (see also Figure 1) was observed. The criterion used to measure the radiosensitivity was the percentage of embryonic lethality (= number of eggs from which no larvae hatched/number of eggs irradiated). Cytological analysis showed that the radioresistant cells are in inter-/pro-phase, the most sensitive cells in ana-/telophase. Recently LEUTHOLD³ irradiated cleavage stages obtained from stocks with variable chromosome numbers. He found that radiosensitivity is positively correlated with the amount of chromosomal material irradiated per cleavage nucleus. This indicates that embryonic lethality is the consequence of radiation-induced chromosome lesions. MATTER⁴ postulated that part of the stage-dependent changes in sensitivity results from a variation in the concentration of free SH groups within the chromosomes. The basis of the remaining variation is not yet clear.

For some cell types it is known that the oxygen consumption changes from stage to stage during the cell cycle (for review see MAZIA⁵). It was shown by KIHLMAN⁶ that active respiration decreases the oxygen concentration within the cell nucleus compared to the oxygen concentration present outside the cell. Stage-dependent variations in the respiratory activity could therefore lead to variable oxygen concentrations in the radiosensitive structures during the cell cycle. Therefore in experiments in which exposure to X-rays is performed in a normal air environment at least part of the sensitivity differences between stages might result from variation in the oxygen content within the radiosensitive structures. Assuming this hypothesis to be true for *Drosophila* eggs, for which the radiosensitive structures during cleavage are chromosomes, we predict that the relative radiosensitivities of different mitotic stages should differ, depending on whether radiation is given under oxic or anoxic conditions. Consequently

¹ F. E. WÜRGLER, H. ULRICH and A. SCHNEIDER-MINDER, in *Repair from Genetic Radiation Damage*, (Ed. F. H. SOBELS; Pergamon Press, London 1963), p. 101.

² F. E. WÜRGLER, H. ULRICH and H. W. SPRING, *Experientia* 24, 1082 (1968).

³ U. LEUTHOLD, *Mutation Res.* 14, 65 (1972).

⁴ B. E. MATTER, *Mutation Res.* 70, 567 (1970).

⁵ D. MAZIA, in *The Cell* (Ed. J. BRACHET and A. MIRSKY; Academic Press, New York and London 1961), vol. 3, p. 77.

⁶ B. A. KIHLMAN, *Expl. Cell Res.* 14, 639 (1958).

Experimental data on embryonic lethality (given in %) observed after X-irradiation of early *Drosophila* embryos in air or nitrogen with 800 R

Age at irradiation (min)	Irradiation in air			Irradiation in nitrogen		
	Eggs tested	Eggs killed	%	Eggs tested	Eggs killed	%
9.5	77	75	97.40	110	82	74.54
10.5	103	83	80.58	114	75	65.79
11.5	74	56	75.68	94	54	57.45
12.5	77	49	63.64	—	—	—
13.5	111	61	54.95	89	30	33.71
14.5	73	41	56.16	82	26	31.71
15.5	—	—	—	113	40	35.40
16.5	98	66	67.35	82	31	37.80
17.5	—	—	—	80	36	45.00
18.5	61	53	86.88	91	56	61.54
19.5	83	79	95.18	74	54	72.97
20.5	96	93	96.88	70	58	82.86
21.5	87	81	93.10	109	75	68.81
22.5	73	64	87.67	59	26	44.07
23.5	46	29	63.04	66	26	39.39
24.5	70	57	81.43	39	21	53.85

The percentages of embryonic lethality are not corrected for spontaneous lethality. This lethality was found to be 6.35%. The nitrogen treatment itself is not toxic.

we expect a variation of oxygen enhancement ratios (OER = ratio between the effect found in the presence and in the absence of oxygen) with the course of the mitotic cycle.

We tested this prediction experimentally using egg samples collected in 3-minute collection periods. All samples were obtained from the same population of flies (stock Berlin wild) and irradiated under identical temperature and humidity conditions (WÜGLER, ULRICH and SPRING²). The samples were numbered continuously, the odd numbered samples were irradiated in air and the even numbered ones were treated for 1 min before and during the exposure with nitrogen (Nachgereinigter Stickstoff; Sauerstoff und Wasserstoffwerke Luzern). WÜGLER and ULRICH⁷ have shown that already a 15-second long nitrogen pretreatment is sufficient to achieve complete anoxia. Humidified gas streams of 3 l/min were used. In order to avoid changes in the stage composition of the egg samples during irradiation 800 R of 50 keV X-rays were applied within only 5 sec. With both treatments egg samples of an average age ranging from 9.5 ± 1.5 to 24.5 ± 1.5 min were

used. The nuclear division stages treated in such egg samples of different age are indicated in Figure 1. The results are given in the Table. The percentages of embryonic lethality are plotted semilogarithmically in Figure 1. For the rationale of the use of a semilogarithmic plot see e.g. ELKIND and WHITMORE⁸. In order to facilitate the comparison of the results obtained in the air series with those of the nitrogen series, the values for the nitrogen series were doubled as shown by the closed circles in Figure 1. These calculated values are very close to those found experimentally after irradiation in air. No systematic variation of the sensitivity pattern is detectable between the effect observed in air or nitrogen. Hence one would assume that for all stages treated, irradiation in air is about twice as effective as in nitrogen. The maximum oxygen enhancement ratio is already reached with a gas mixture of 10% O₂ + 90% N₂ (FINSINGER⁹, see also Figure 2). The factor 2 obtained from the comparison of the effects in nitrogen and air is therefore a rough estimate of the OER. This value is comparable to the value reported for 10–20-minute-old eggs (so-called zygotes; WÜGLER¹⁰). The im-

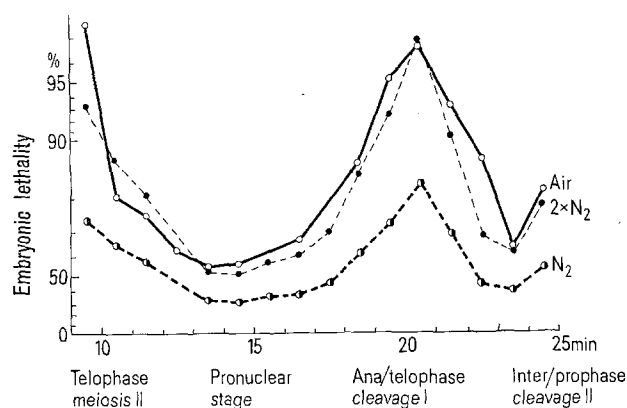


Fig. 1. Stage-dependent variation of radiosensitivity in early *Drosophila* embryos irradiated either in air or in nitrogen. For comparison, the data for irradiation in nitrogen were doubled (closed circles).

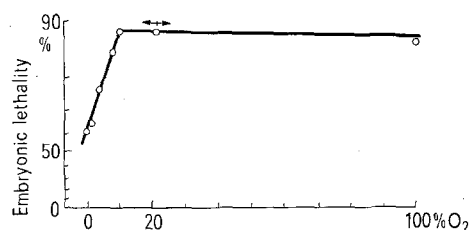


Fig. 2. Dependence of X-ray-induced embryonic lethality on the oxygen concentration in the gas surrounding the eggs (FINSINGER⁹). The arrow indicates that small changes in the oxygen-concentration due to variable rates of respiration during the cell cycle do not lead to measurable changes in the radiation response.

⁷ F. E. WÜGLER and H. ULRICH, *Experientia* 28, in press (1972).

⁸ M. M. ELKIND and G. F. WHITMORE, *The Radiobiology of Cultured Mammalian Cells* (Gordon and Breach, New York 1967).

⁹ F. X. FINSINGER, *Vjschr. Naturforsch. Ges. Zürich* 109, 175 (1964).

portant result with respect to radiobiology of early cleavage stages is the demonstration that differences in oxygen concentration within the cell nucleus during the progression through the cell cycle do not contribute to the variation of radiosensitivity.

On the other hand, these tests do not exclude the possibility that the oxygen concentration within the nucleus changes during the cell cycle. Figure 2 shows the dependence of the X-ray-induced rate of lethality on the external oxygen concentration as demonstrated by FINSINGER⁹ for zygotes. As is indicated by the arrows, small respiration-dependent changes in the oxygen concentration within the cell nucleus cannot influence the radiosensitivity to a measurable extent¹¹.

Zusammenfassung. *Drosophila*-Embryonen zeigen eine mit dem Ablauf der Furchungsteilungen korrelierte Variation der Strahlenempfindlichkeit. Für alle Mitosesta-

dien wurde eine Sauerstoff-Erhöhrungsrate von rund 2 festgestellt. Dies zeigt, dass eine vermutete atmungsbedingte Variation des Sauerstoffgehaltes in den Zellen nicht am Zustandekommen der Empfindlichkeitsvariation beteiligt ist.

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¹⁰ F. E. WÜRGLE, Rev. Suisse Zool. 67, 295 (1960).

¹¹ Work supported by Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung.

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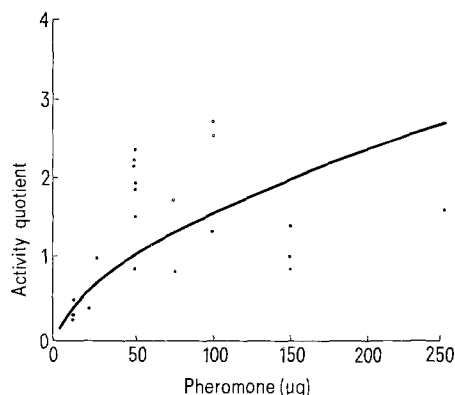
Attraction of the Male House Fly to Cuticular Hydrocarbons and Feces of Several other Dipteran Species

The male house fly, *Musca domestica* L., responds to a pheromone¹ which was found in hydrocarbons of the cuticle and feces of sexually mature females^{2,3}. This pheromone was isolated and chemically identified as *cis*-9-tricosene⁴. In an earlier study, it was shown that male house flies also were attracted to the feces of the stable fly, *Stomoxys calcitrans* (L.)⁵. This suggested that cross attraction might occur among various species in the family Muscidae. Cross attraction has been demonstrated conclusively in the order Lepidoptera where several species and families utilize the same pheromone⁶⁻⁹. Generally, where several sympatric species use the same sex pheromone, the presence of additional pheromones may be postulated to explain reproductive isolation and to reduce meaningless communicative signals.

The behavior of Muscid flies in a bioassay system makes it difficult to measure responses to pheromone. Further studies on other species of flies will require investigating their behavioral patterns before implementing methods for pheromone assays. Because our house fly assay system provides reliable data, it was considered important to con-

duct preliminary tests on the attractiveness of cuticular hydrocarbons and feces from other Dipteran species in the male house fly bioassay system even though in-depth studies are not planned at this time. This communication reports results of these preliminary tests which may be useful to others currently conducting Dipteran pheromone studies.

The bioassay techniques and olfactometer design have been described in detail elsewhere^{2,3,10}. For these tests, 300 newly emerged male house flies were placed in each of 4 olfactometers maintained at 28°C, 60% R.H., and were held until they were 2 or 3 days old before bioassaying. The cuticular lipids were obtained as previously described and the hydrocarbons were isolated by hexane elution from silicic acid³. Aliquots of the eluate were concentrated in a rotary evaporator and the concentrate was applied to filter paper for bioassay. Fecal samples were collected on paper towels or filter paper and tested without further purification. Each test material was bioassayed as previously described using Edamin (hydrolyzed milk protein) as an internal standard and 60 mg of crude fecal lipid as an external standard. Activity quotients² were used as indices of attractiveness. The activity quotient as a measure of sensory response is demonstrated in the Figure for the natural house fly pheromone, *cis*-9-tricosene. These data confirm the proper function¹¹ of this type of olfactometer



Relationship of quantity of *cis*-9-tricosene to attraction (expressed as A.Q.) of the male house fly (power curve $Y = aX^b$; $a = 0.10$, $b = 0.59$; $r = 0.69$; $p < 0.01$ at d. f. = 20).

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