

column according to BISHOP, PANDYA and KING⁸. Diglycerides, triglycerides, hydrocarbons and free fatty acids were estimated by the procedures of ELSBACH⁹.

Table I gives the gross composition of various lipids. Total lipids were about 7.8% of dry wt. of the cells out of which phospholipids were the major constituents (55–58%). Amount of phospholipids in total lipids of human leukocyte¹⁰ of undifferentiated morphological type is 10% less compared with that of alveolar macrophages, while that of rabbit polymorphonuclear leukocytes⁹ phospholipids is slightly more (about 3%) than that of macrophage phospholipids. Free cholesterol content was about 7% of the total lipid content and a similar amount of esterified cholesterol was also present. Results indicate that the composition of alveolar macrophage lipids differ from that of lung lipids¹¹.

Table I. Lipids of alveolar macrophages

	Dry wt. of cells (%)
Total lipids	7.84 ± 2.6
Phospholipids	54.98 ± 3.10
Neutral lipids	
Cholesterol, free	6.53 ± 1.3
Cholesterol, esterified	7.96 ± 1.5
Triglycerides and free fatty acids	21.18 ± 2.4
Hydro carbons	2.25 ± 0.5
Diglycerides and others	6.39 ± 1.1

Table II. Ubiquinone (coenzyme Q) content of guinea Pig alveolar macrophages

Dry wt. of tissue (mg)	Method of extraction	Ubiquinone (μmoles/g dry wt.)
1798	n hexane	0.31
1144	n hexane	0.38
1550	methanol	0.27
953	methanol	0.33

The lipids were fractionated by alumina or silica column chromatography⁸. The 4% and 6% ether in light petroleum fraction gave a typical spectrum of ubiquinone with a peak at 275 nm (in ethanol). On reduction with sodium borohydride, 275 nm peak disappeared and a new peak at 291 nm was emerged. It is of significance to note that about 0.32 μm moles/g dry wt. of ubiquinone was present in the guinea-pig alveolar macrophages (Table II). This indicates that alveolar macrophages may have a well developed oxidative system. In the cells which have well defined mitochondria like leukocytes and alveolar macrophages, presence of ubiquinone is expected but has not been detected or isolated so far. The present report of separation of ubiquinone from alveolar macrophages reinforces the above view-point. The erythrocytes do not contain detectable amount of ubiquinone¹². Isoprenologue of ubiquinone was identified by thin layer chromatography as described by PANDYA et al.¹³. It was found to contain ubiquinone-10. IR-spectrum of petroleum fraction of the silicic acid column chromatography indicates absorption maxima for the presence of long chain monohydric isoprenoid alcohol (like dolichol or solanesol).

Zusammenfassung. Lipidanalyse von Meerschweinchen-Makrophagen.

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Nitrogen Pretreatment Period Required for Complete Elimination of the Oxygen Effect in X-rayed *Drosophila* embryos

With nearly all biological test systems with which radiobiological experiments are performed, an oxygen effect is observed. By 'oxygen effect' we mean that irradiation of a test system with a given dose in the presence of oxygen is more efficient than the same dose given in nitrogen, with respect to the amount of damage induced. The maximum enhancement of the radiation response due to oxygen is called the oxygen enhancement ratio (OER). The OER is calculated as the ratio 'effect in the presence of oxygen/effect in the absence of oxygen'.

The anoxic condition is usually achieved by treatment of the cells or organisms with nitrogen. For the complete elimination of the oxygen effect and in consequence for the determination of the OER the length of the pretreatment period with nitrogen needed for a particular

test system is critical. For *Drosophila* embryos a pretreatment period of 1 min has been claimed to be sufficient (ULRICH¹, WÜRLER², FINSINGER³, MATTER⁴). Experimental evidence on which this statement is based has never been published.

We describe here experimental data indicating that as short a time as 15 sec is sufficient to remove all oxygen from the radiosensitive structures of the cells. For our experiments we used 15 ± 5 min old eggs which were

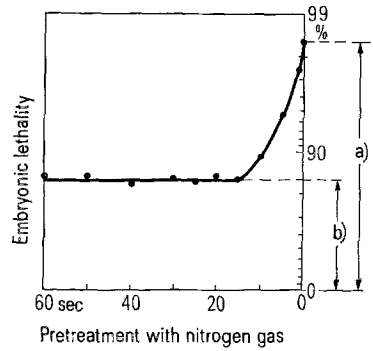
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collected, using a simplified modification of the method described by WÜRGLER, ULRICH and SPRING⁵. In these egg samples we find predominantly pronuclear (= zygote) stages and stages of the first cleavage division. From the embryological point of view these are early embryonic stages. The samples were pretreated with purified nitrogen (Sauerstoff- und Wasserstoff-Werke Luzern) for variable periods of time ranging from 0 to 60 sec in a plexiglas



Embryonic lethality (ordinate) observed after irradiation of *Drosophila* embryos pretreated with nitrogen gas during pretreatment periods of variable duration (abszissa). The arrows a) and b) represent the values used for the calculation of the oxygen enhancement ratio.

Effect of nitrogen pretreatment on the rate of embryonic lethality after X-irradiation of 15 ± 5 min old *Drosophila* embryos with 2000 R

Duration of pretreatment (sec)	No. of embryos		Embryonic lethality (%)
	Tested	Dead	
0 (= air)	635	625	98.40
1	439	429	97.72
5	747	708	94.78
10	625	557	89.12
15	832	701	84.25
20	976	829	84.94
25	580	486	83.79
30	835	702	84.07
40	384	318	82.81
50	366	311	84.97
60	517	439	84.91
average 15 to 60	4490	3786	84.32

chamber of 48 cm³ with a humidified gas stream of 3 l/min. A 2000 R dose of 50 keV X-rays was applied within 1 sec. The Table gives the actual data. The percentages of embryonic lethality (= 100 × number of eggs from which no larva hatched/number of eggs irradiated) are plotted semilogarithmically in the Figure. For the mathematical rationale to use semilogarithmic plots see ELKIND and WHITMORE⁶. Since each point in the Figure represents one point of an exponential dose response curve (WÜRGLER²) and since oxygen was shown to be a dose modifying agent (READ⁷) we find an OER of: $OER = a/b = -\ln(1-0.9840)/-\ln(1-0.8432) = 2.2$. For the meaning of a) and b) see the Figure. This value is in good agreement with corresponding values published earlier (WÜRGLER², FINSINGER³, MATTER⁴).

The results indicate that, with our technique, a pretreatment period of only 15 sec is sufficient to achieve complete anoxia. It is interesting that BELL and ROACH⁸ found a pretreatment period of the same order of magnitude (20 sec) for cultured mammalian cells. In contrast, if cells in adult flies are to be irradiated in anoxic condition, pretreatment periods up to 30 min must be used (SOBELS⁹).

Two phenomena might contribute to the fact that such an extremely short pretreatment period is sufficient to achieve anoxia in individual cells: 1. Quick diffusion of oxygen out of the cell due to the gradient of oxygen concentration achieved by the nitrogen gas surrounding the cells and 2. rapid consumption of the available oxygen by cellular respiration¹⁰.

Zusammenfassung. Zur vollständigen Ausschaltung des Sauerstoff-Effektes bei der Bestrahlung von *Drosophila*-Embryonen genügt eine 15 Sekunden dauernde Vorbehandlung mit Stickstoff.

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Beziehungen zwischen laccase- und peroxidaseartigen Isoenzymen von Basidiomyceten

Untersuchungen von LYR¹, KOENIGS² und neuerdings HOLUBOVÁ-JECHOVÁ³ ergaben den Hinweis, dass bei Basidiomyceten neben Laccasen und Tyrosinasen auch Peroxidasen vorkommen. Diese Ergebnisse beruhen auf Beobachtungen an Agarkulturen oder Myzelhomogenaten (Peroxidaseaktivität = Steigerung der Phenoloxidasenaktivität nach Zusatz von H₂O₂). Wir versuchten nun für diese Effekte verantwortliche Isoenzyme mittels Elektro-

fokussierung (IEF)^{4,5} aufzutrennen. Unsere Resultate zeigten, dass eine Steigerung der Phenoloxidasenaktivität in Rohextrakten, Kulturfiltraten oder Agarkulturen durch Zusatz von H₂O₂ meist nicht auf besondere Peroxidasen, sondern auf die Eigentümlichkeiten der vorhandenen Laccasen zurückgeht. Dies ergaben Untersuchungen an Myzelextrakten und Kulturfiltraten von über 30 Pilzen. Ein detaillierter