system, since the liver plays an important role in endotoxicosis[8] and endotoxin depletes carbohydrate reserves first of all from the liver[4], and reverses gluconeogenesis; c) glucose feeding was prolonged until the 16th hour after endotoxin challenge, contrary to other studies cited above with only 1 or 2 injections and small quantities of glucose.

Materials and methods. Swiss, male, albino mice (24 ± 2 g) were housed on laboratory food and water ad libitum; food was withdrawn at the beginning of the experiments. To determine lethality, mice were injected with 500 µg of E. coli 026:B6 endotoxin (Difco, lot 586787) i.p. and the survival was recorded 48 h later. The LD_{50} was calculated according to Reed and Muench[9]. Glucose (5%), sodium chloride (0.9%), or water, in 0.5 ml, was given by stomach tube 1 h before and 1, 6, 11 and 16 h after endotoxin challenge.

Liver glycogen levels were determined according to the method of Kemp and Kits van Heijningen[10] as adapted previously[11]. The glycogen level is expressed as mg/100 mg fresh liver weight. The results were evaluated by the Student t-test and the chi square test. A SE was calculated for all mean values.

Results and discussion. Data in the table show that the administration of a total of 125 mg glucose (5% solution) by gavage (25 mg per dose, 1 h before and 1, 6, 11, 16 h after endotoxin challenge) did not alter endotoxin lethality. Oral administration of physiological saline was also without effect on the outcome of endotoxin lethality. There was 36%, 40.7% and 40% survival level in the water, physiological saline and 5% glucose treated groups, respectively.

Influence of glucose or saline feeding on endotoxin lethality in normal mice

Treatment	Living/Total (48 h)	Percent survival	Statistics
1. Water	9/25	36	
2. Physiological saline	11/27	40	2 VS 1 NS
3. 5% glucose	10/25	40	3 VS 1 NS

NS = not significant.

Data in the figure clearly show that liver glycogen both in the water-treated and physiological glucose-treated groups was significantly lower within 4 h after endotoxin challenge, and continued so until a minimal carbohydrate level was achieved in both groups. The liver glycogen level did not increase in response to glucose feeding in endotoxin-challenged groups.

These results support earlier observations[4-6] obtained in endotoxin hyperreactive animals, with i.v. glucose administration, that exogenous glucose supply does not influence the outcome of endotoxin lethality. As in earlier studies[4,5] with parenteral glucose administration, exogenous glucose given by stomach tube also failed to prevent glycogen depletion during endotoxicosis (figure), either because it was metabolized and/or converted to muscle glycogen since mucsle glucogenesis is not impaired in endointoxicated animals[4]. It may be that liver glycogenesis is controlled at 2 levels; glucose-induced cycle can only proceed under normal conditions but cortisone-induced phase can proceed just as well in endotoxin poisoned animals as in normal mice, although cortisone-sparing of liver glycogen levels could only be secondary to protection against endotoxin lethality. Finally, the primary event in endotoxin death still remains elusive.

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Influence of anaesthesia by carbon dioxide and ether on locomotor activity in *Drosophila melanogaster*

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Summary. Locomotor activity of Drosophila melanogaster was investigated in 2 recently caught unrelated wild stocks. It is strongly affected by anaesthesia with either carbon dioxide or ether. Both anaesthetics had opposite effects. Ether produces a longlasting decrease in activity. Carbondioxide causes an increase of locomotor activity, but smaller and of shorter duration than the effect of ether.

Research on activity of Drosophila has shown that, it is a complex character, which is under control of both genetic and environmental factors[1-5]. It could be expected that anaesthetics, used to immobilize flies, would affect the locomotor activity in later life. During experiments on locomotor activity, we obtained evidence that these effects could be considerable.

Therefore an experiment was started to investigate the long-term effects of both ether and CO_2 on this character. The locomotor activity of 2 wild strains of Drosophila melanogaster, recently collected in Dahomey (now Benin) and Spain (La Mancha) was measured in an apparatus that was especially constructed for this purpose[6]. It is a modification of the apparatus, used by Ewing[1], and consists of a row of 20 vials, which can be transversed in one direction only.

The mean activity score was calculated as the mean number of vials the flies have visited after 20 min. The activity tests were performed at 20 ± 1 °C. Virgin flies were collected with either ether or CO_2 and tested at ages of 30, 300 and 450 h. Some of the flies of the 300 and 450 h old flies were submitted to a subsequent anaesthesia with either CO_2 or ether. The ether was tested on the presence of peroxides. The results were submitted to an analysis of variance. No interactions between the first

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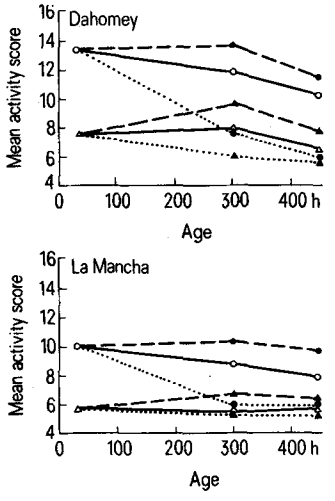
Mean activity scores of flies at ages of 30, 300 or 450 h (sexes pooled)

Age	1st anaesthetic	2nd anaesthetic	Dahomey	N	La Mancha	N
30	Ether	–	7.64	203	5.72	201
	CO_2	–	13.29	186	9.92	199
300	Ether	Ether	6.09	196	5.29	181
	Ether	CO_2	9.53	208	6.52	190
	Ether	–	7.57	201	5.32	191
	CO_2	Ether	7.43	187	5.71	199
	CO_2	CO_2	13.48	188	10.15	184
	CO_2	–	11.61	185	8.77	178
450	Ether	Ether	5.62	197	5.14	186
	Ether	CO_2	7.70	184	6.00	182
	Ether	–	6.37	149	5.76	146
	CO_2	Ether	5.86	188	5.80	181
	CO_2	CO_2	11.23	186	9.56	187
	CO_2	–	10.11	149	7.86	174

N = Number of flies.



Effects of anaesthesia with CO_2 and ether on locomotor activity of 2 stocks of Drosophila melanogaster. Circles represent flies collected with CO_2, and triangles flies collected with ether. Solid symbols stand for subsequent anaesthesia. Dashed lines: subsequent anaesthesia with carbondioxide. Dotted lines: subsequent anaesthesia with ether.

and second treatment were found ($p > 0.05$). In the table and the figure we averaged the mean activity scores of males and females, because no significant differences were found between both sexes ($p > 0.25$). The most striking result from this experiment, which is highly significant ($p < 0.001$), is the overall activity-decreasing effect of ether anaesthesia throughout the experiment. The fact that ether has such a longlasting effect does not agree with the impression of many Drosophila-workers, who think that ether effects have disappeared after 24 h[7]. Contrary to ether, CO_2 causes an increase in activity, but this effect does not seem to last so long. Perron et al.[8] found some toxic effects of CO_2 in D. melanogaster on physiological characters but only when very young flies were treated over a long period.

In accordance with the results of Hardeland and Stange[5], we did not detect age effects on the locomotor activity ($p > 0.10$). There is a difference in activity between the 2 stocks: flies of the Dahomey stock are more active than La Mancha flies ($p < 0.01$). This is probably correlated with the difference in body-size.[9]

The physiological bases of the anaesthetic effects are not known. King and Wilson[10] suggest that ether may upset the phosphorus turnover. Wigglesworth[11] proposed that oxygen lack caused by CO_2-anaesthesia prevents the oxidation of acid metabolites produced by the activity of the insect. Hardeland and Stange[5] pointed out that there could be a connection between locomotor activity and the activity of cytochrome-c-oxidase. We have shown that NADH-dehydrogenase activity in the mitochondria is highly correlated to locomotor activity (Thörig et al., unpublished, Scharloo et al., in press). Perhaps it can be supposed that the anaesthetics affect these enzymes by altering the structures of membranes[12].

This suggests the possibility of interference of the anaesthetics with the energy metabolism of the insect. It is clear that ether produces not only big, but also long, perhaps livelong effects on the locomotor activity in Drosophila melanogaster. Therefore it should be recommended to minimize the use of anaesthetics without careful analysis of their effect, especially when working on behavioural traits.

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Effect of S-adenosyl-L-methionine[1] on ethynylestradiol-induced impairment of bile flow in female rats

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Summary. Prevention by S-adenosyl-L-methionine (SAMe) of the bile flow reduction induced by ethynylestradiol (EE) is demonstrated by comparing the flow rate and the bile salt concentration of bile in EE-treated animals with that in animals given both EE and SAMe.

Changes in bile flow and composition have been shown in the rat following EE-administration[3,4], and have been correlated with the impairment of the system responsible for the secretion of the bile salt-independent fraction of the canalicular bile[5]. Moreover, it has been reported by various authors[6,7] that O-methylated derivatives of 2-hydroxyestrogens constitute the major fraction of urinary estrogens.

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