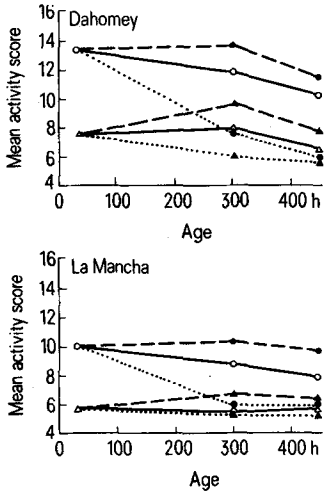
Mean activity scores of flies at ages of 30, 300 or 450 h (sexes pooled)

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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Since SAMe is the methylating agent in the enzymatic methylation by catechol-O-methyl transferase[8–10], the effect of SAMe administration on the bile flow reduction by EE was tested.

Materials and methods. Female Sprague-Dawley rats weighing 200 ± 10 g were used for the experiments, which consisted in measuring the bile flow and determining the bile salt concentration in 5 groups of animals treated as follows: 3 groups were given for 3 days by gastric intubation 2.5 mg/kg of EE in 2% gum arabic in a volume of 10 ml/kg. To the animals of 2 of these groups, SAMe was given i.m. 3 times a day for 3 days at doses of 10 and 25 mg/kg respectively, the first dose being injected 30 min before the first EE-administration and the last one 1 h before the bile collection. 2 control groups were treated as follows: the 1st group received 10 ml/kg of 2% gum arabic by gastric intubation; the 2nd one received gum arabic in the same way and SAMe 25 mg/kg by i.m. injection 3 times a day for 3 days. All the animals were fasted 12 h before bile collection, which was done under urethan anesthesia obtained with 1 ml/kg of a 50% urethan solution administered i.p. Bile salt concentration was determined by the enzymatic method described by Talalay[11] and data were analyzed by Student's t-test.

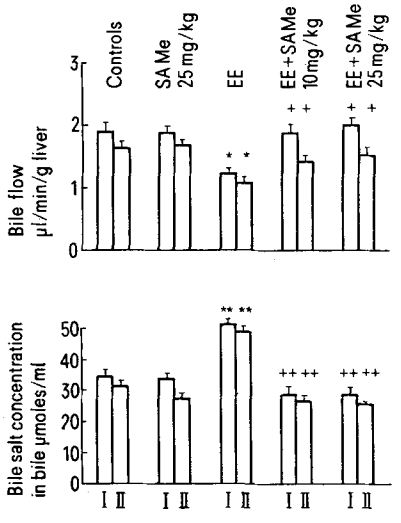

Fig. 1. Effect of SAMe-administration on bile flow and bile salt concentration in rats treated with EE. I, Collection time from 0 to 30 min; II, collection time from 30 to 60 min. Values are expressed as mean ± SE for 6 rats. * $p < 0.05$ in comparison with control rats. + $p < 0.05$ in comparison with EE-treated rats. ** $p < 0.01$ in comparison with control rats. ++ $p < 0.01$ in comparison with EE-treated rats.

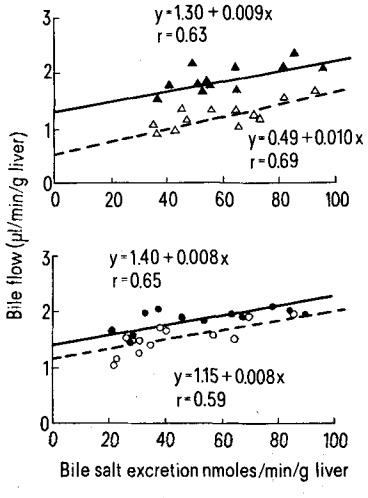

Fig. 2. Relationship between bile flow and bile salt excretion. Regression lines calculated by the method of the least squares, where: y = mx + c; y = canalicular bile flow and x = rate of bile salt excretion; r = correlation coefficient. ▲—▲ control rats, △--△ EE-treated rats, ○--○ EE + SAMe 10 mg/kg, ●—● EE + SAMe 25 mg/kg.

Results. As shown in figure 1, bile flow was slower in the EE-treated group than in control rats ($p < 0.05$) up to 60 min after starting of bile collection. The administration of 10 or 25 mg/kg of SAMe in addition to EE was capable to restore the bile flow to the control values. The bile salt concentration was higher ($p < 0.01$) in the EE-treated animals when compared with either the control animals or those receiving EE and SAMe, although the amount excreted did not change significantly. The relationship between bile flow and bile salt excretion is shown in figure 2. In order to compare the bile salt-independent fraction of the bile in the different groups, the best fitting lines and the intercepts on the y-axis were calculated by regression analysis of the experimentally obtained values. A significant difference ($p < 0.01$) was observed when the bile salt-independent fraction calculated for EE-treated animals (0.49 µl/min/g liver) was compared with that of control animals (1.30 µl/min/g liver). Values of 1.15 and 1.40 µl/min/g liver calculated for animals treated respectively with 10 and 25 mg/kg SAMe in addition to EE were statistically indistinguishable from those of control animals but did differ significantly from those of EE-treated rats ($p < 0.01$).

Considering the bile flow during the first 30 min of bile collection as 100%, the bile salt-independent fraction of bile water represents 66% of the total bile flow in the control rats, 39.2% in the EE-treated rats, 60 and 68% respectively in the animals given 10 and 25 mg/kg SAMe in addition to EE.

Discussion. Reduction of bile flow after EE-treatment has been observed by other authors[3,5]. Moreover, it has been postulated that this might be associated with alteration of the canalicular water secretion into bile independently from the excretion of bile salts[5]. The results reported above are in good agreement with these observations, since significant differences were observed in bile flow for EE-treated with respect to control animals, whereas bile salt excretion was unmodified. On the other hand, bile flow appears unmodified in rats given SAMe in addition to EE with respect to controls. Moreover, the bile salt-independent fraction of bile water which is also modified by EE-treatment shows values similar to controls in animals treated with EE and SAMe.

Since it is known that no methylated steroid was irreversibly bound to the microsomal proteins in rat liver[12], it might be postulated that SAMe-treatment reduces this binding, perhaps by favouring the enzymatic methylation of the catechol estrogens by the catechol methyl transferase[8–10].

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