of 20 eggs each were injected with 0, 1, 10, 100, 1000 or 10,000 µg/egg CPZ at 96 h of incubation. When hatched, the chicks were exposed to the following behavioral tests: imprinting (beginning at 3 days of age); open field (14 days of age); conditioned avoidance response to light onset (21 days of age); position habit in a T maze for a food reward (21 days of age). All chicks that survived to hatching were subjected to a standardized autopsy procedure[22,23].

Results. The lethal dose 50%, as determined by the exact probit method[24], was 4000 µg/egg. The groups treated with 1, 10, 100 µg CPZ took fewer trials to reach criterion on the T maze than the control group, while the 1000 and 10,000 µg groups took more trials (Kruskal-Wallis, 0.05 confidence level)[25]. While not statistically significant, the following observations also suggest long term alterations in behavior. The drug-treated chicks tended to have shorter latencies in an imprinting situation, less activity in an open field apparatus, and tended to require more trials to criterion in the conditioned avoidance problem. 17% of all of the drug-treated chicks that survived to hatching showed a 'curled toe' anomaly. The 'curled toe' could be relatively mild and involve 1 or 2 toes on 1 foot, or relatively severe, as shown in the figure, and involve all of the toes on 1 or both feet. The effective dose 50% for 'curled toes' is 88 µg/egg as determined by the exact probit method[24].

Discussion. The 'curled toe' anomaly was not reported by other investigators who exposed chick embryos to CPZ[26,27]. However, the 'curled toe' anomaly is quite similar to that reported for chicks born of riboflavin-deficient mothers[28]. It seems likely that the curled toe is a physiological rather than strictly an anatomical anomaly since the 'curled toe' released to some extent during Nembuthal anesthesia (1.0 mg/kg). While great caution should be exercised in extrapolating from animal studies to humans, it is clear that prenatal exposure to CPZ is associated with long term behavioral alterations[29–36]. It is also clear that prenatal exposure to CPZ can be associated with extrapyramidal dysfunction in humans[13–15]. Since the animals in this study also demonstrated behavioral as well as a potential neurological anomaly, it would be important to reconsider the administration of CPZ to human females of childbearing age and in particular, as an antiemetic during the critical first trimester of pregnancy.

23 E. J. Wortley, in: Poultry Diseases. Orange Judd Co., New York 1915.
24 M. G. Natrella, in: Experimental Statistics. Government Printing Office, Washington 1963.
25 R. E. Kirk, in: Experimental Design: Procedures for the Behavioral Sciences. Brooks/Cole, Belmont, California 1968.
26 A. Vernadakis, Brain Res. *12*, 223 (1969).
27 Z. B. Miller and M. C. Pasciuto, Proc. Soc. exp. Biol. Med. *14* (1963).
28 A. E. Schumacher and G. H. Heuser, Poultry Sci. *18*, 369 (1939).
29 D. R. Hoffeld, J. McNew and R. L. Webster, Nature *218*, 357 (1968).
30 D. R. Hoffeld and R. L. Webster, Nature *209*, 1070 (1965).
31 D. R. Hoffeld, R. L. Webster and J. McNew, Nature *215*, 182 (1967).
32 N. Murai, Tohoku J. exp. Med. *89*, 265 (1966).
33 J. Werboff and J. Haulena, Exp. Neurol. *6*, 263 (1962).
34 J. Werboff and R. Kesner, Nature *197*, 106 (1963).
35 V. Hlavackova and H. Reutt, Sb. lék. *65*, 159 (1963).
36 R. E. Jewett and S. Norton, Exp. Neurol. *14*, 33 (1966).

Toxicity of ouabain on Drosophila melanogaster

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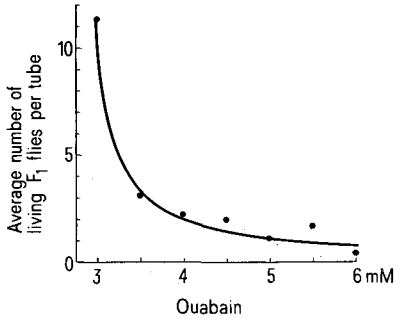
Summary. Ouabain, also called g-strophantin, is an inhibitor of the Na^+/K^+-activated plasma membrane ATPase. Treatment of Drosophila melanogaster with 3–6 mM of this substance leads to a markedly reduced survival of the flies.

Recently, the use of ouabain as a selective agent in mammalian cell cultures has been described by some authors, and they were able to show that this compound can be applied in an in vitro system for mutagenicity testing[1–3]. In an attempt to use ouabain resistance as a selective system not only for cells in culture but also for whole animals, we tested the toxicity of the compound with adult Drosophila flies and the development of their progeny. The used strain was Berlin wild (+K). The animals were fed with a cornmeal-agar-syrup medium containing different concentrations of ouabain. For each concentration 10 test tubes were used. 3 pairs of flies were put into each tube. After a treatment period of 6 days at 28 °C (this temperature was chosen to shorten the usual generation time of 14 days at 26 °C), the parent flies were removed. After an additional treatment period of 6 days, the F_1 progeny was scored.

At a low concentration, the toxicity of ouabain increases with increasing concentration (table). At higher concentrations, a saturation effect is clearly demonstrated in the figure where the average number of living F_1 animals per tube is plotted against the ouabain concentration. An exact estimation of the dead flies in the tubes was not possible. With the results described, it is possible to test



Survival of the progeny after ouabain treatment.

1 R. M. Baker, D. M. Brunette, R. Mankovitz, L. H. Thompson, G. F. Whitmore, L. Siminovitch and J. E. Till, Cell *1*, 9 (1974).
2 C. F. Arlett, D. Turnbull, S. A. Harcourt, A. R. Lehmann and C. M. Colella, Mutation Res. *33*, 261 (1975).
3 P. J. Davies and J. Parry, Genet. Res. *24*, 311 (1974).

Effect of ouabain on Drosophila melanogaster

Concentration of ouabain (mM/l)	Dead parent flies (out of 30) in 10 tubes after 6 days		Living F_1 flies in 10 tubes after 12 days		
	Males	Females	Males	Females	Total
0	2	–	266	288	554
3.0	–	13	72	42	114
3.5	–	9	12	19	31
4.0	–	17	12	10	22
4.5	–	13	11	9	20
5.0	–	18	6	5	11
5.5	2	21	7	10	17
6.0	2	21	2	2	4

if the induction of resistance against ouabain can be achieved in Drosophila. Some questions remain open. It is not clear if the decreased survival of the progeny after ouabain treatment is the result of an effect on the germ cells of the male parents, on the germ cells of the female parents or on the development of the progeny itself. There might be a more complex effect, too. This question might be answered by testing the fertility of adult flies after ouabain treatment.

A problem that should also be mentioned is the marked difference of the toxicity of the substance against adult flies. Female flies are killed by ouabain to a much higher extent than male ones. This might be overcome by taking an increased number of females in the test, but further investigations must clarify the sex difference of Drosophila melanogaster against ouabain.

Enhancement of barium- and cesium-induced adrenal catecholamine release by lidocaine[1]

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Summary. Catecholamine release evoked from isolated perfused bovine adrenals by Ba^{+2} or Cs^+ is enhanced by lidocaine or by a calcium-free medium. The action of Cs^+ therefore differs from that of K^+ or Rb^+ in adrenal medulla. Divalent and monovalent metallic cations of relatively large atomic weight like Ba^{+2} and Cs^+, probably penetrate the cell more easily than small highly charged ions and act intracellularly to cause adrenal catecholamine release. Local anesthetics and calcium-free media may allow greater influx of Ba^{+2} and Cs^+ into adrenomedullary cells.

In contrast to the diminished action of acetylcholine or K^+ in a calcium-free medium, Ba^{+2} is more effective in adrenal medulla in the absence of extra-cellular calcium[2]. Cesium ion is reported[3] to release adrenal catecholamines in a manner similar to K^+, and would appear to have a different mechanism than Ba^{+2} in this tissue. However, the present study shows that the actions of both these ions are enhanced by lidocaine in adrenal medulla and suggests that they have mechanisms in common.

Fresh bovine adrenals were perfused (10 ml/min) with an aerated tris buffered Lockes solution, 22 °C as previously described[4]. Addition of Ba^{+2} to the medium caused catecholamine release (figure 1). The perfusate was assayed for total catecholamines by the colorimetric method of von Euler and Hamberg[5]. When lidocaine, 0.1 mM, was added along with Ba^{+2}, an enhanced response was seen (figure 1). The table shows that adrenal catecholamine release by Cs^+ is also enhanced by 0.1 mM lidocaine. The stimulatory effect of Cd^{+2} on adrenal medulla[6] was not altered by 0.1 mM lidocaine (4 glands). Dose response curves to La^{+3} in 6 pairs of bovine adrenals showed a decreased catecholamine release (20%) when lidocaine, 0.1 mM, was also present but the difference was not statistically significant. Thus enhancement of adrenal catecholamine release by lidocaine occurs with only a few metal ions of relatively large atomic weight and relatively low charge density.

Since local anesthetics block calcium flux across adrenomedullary plasma membranes[7], it is not surprising that barium's action on adrenal medulla is enhanced both by lidocaine and by ommission of calcium from the perfusing fluid. Cesium's effect in rabbit adrenals however is reportedly not enhanced by a calcium-free medium[3]. This seemed illogical so the effect of calcium lack on cesium's action was retested in isolated bovine adrenals. Rubidium was studied for comparison. Adrenal catecholamine release by Rb^+ is largely dependent on extracellular calcium although a

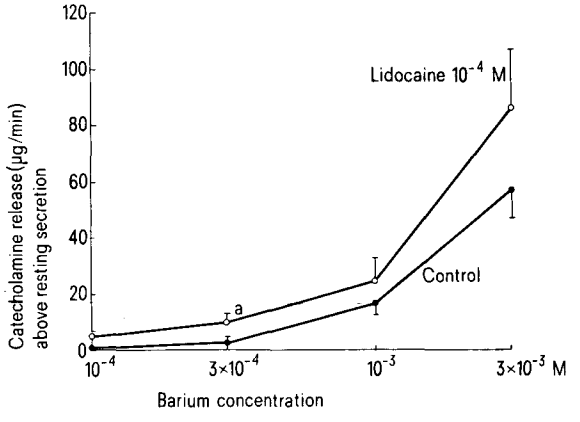

Fig. 1. Enhancement of Ba^{+2}-induced catecholamine release from bovine adrenals by 0.1 mM lidocaine. The barium solutions were infused (10 ml/min) one after the other. Each point is the mean of 4 experiments. a = $p < 0.05$ compared to control. Lidocaine alone has no effect on adrenal catecholamine release.

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2 W. Douglas and R. Rubin, Nature *203*, 305 (1964).
3 M. Sorimachi, Eur. J. Pharmac. *3*, 235 (1968).
4 J. Borowitz, Am. J. Physiol. *22*, 1194 (1971).
5 U. von Euler and U. Hamberg, Acta. physiol. scand. *19*, 74 (1949).
6 D. Hart and J. Borowitz, Archs int. Pharmacodyn. Thér. *209*, 94 (1974).
7 R. Rubin, M. Feinstein, S. Jaanus and M. Raimre, J. Pharmac. exp. Ther. *155*, 463 (1967).