^{3}H-ouabain orally administered, ^{3}H-ouabain absorbed by the gastro-intestinal tract and percent of H^3-ouabain intestinal absorption in guinea pigs 1, 5 and 15 h after administration

	No.	Administered ^{3}H-ouabain		Absorbed ^{3}H-ouabain (dpm/kg)	Absorbed ^{3}H-ouabain (%)
		μg/kg	dpm/kg		
After 1 h	7	250	14,090,000	490,500 ± 36,950	3.54 ± 0.28
	7	500	28,180,000	1,003,000 ± 53,350	3.55 ± 0.19
	7	1,000	56,360,000	2,065,000 ± 169,700	3.66 ± 0.30
After 5 h	7	250	14,090,000	968,200 ± 54,415	6.81 ± 0.38
	7	500	28,180,000	1,941,500 ± 64,293	6.86 ± 0.23
	7	1,000	56,360,000	3,430,000 ± 171,468	6.06 ± 0.30
After 15 h	7	250	14,090,000	1,345,286 ± 94,717	9.71 ± 0.59
	7	500	28,180,000	2,671,500 ± 144,500	9.42 ± 0.49
	7	1,000	56,360,000	5,307,500 ± 358,000	9.40 ± 0.63

administered to the amount of glycoside absorbed to be computed (Figure). The calculation was effected by the straight-line regression method, using an Olivetti P 101 computer. The coefficient of linear correlations was virtually 1, thus demonstrating that the dose/response ratio calculated was linear.

The results of our investigations on guinea-pigs are quite consistent both as regards the amount and the regularity of enteral absorption of ^{3}H-ouabain administered at doses ranging between 250 and 1000 μg/kg. Our data do not agree with those obtained by LAUTERBACH and VOGEL[2] who calculated enteral absorption of ouabain and some other cardiac glycosides by using the technique of HATCHER and BRODIE[1]. They did not find any linear relationship between the quantity of ouabain administered and absorbed. In any case, the method used by LAUTERBACH and VOGEL has recently been demonstrated by VOGEL himself and by BAUMANN[9] to be unreliable.

However, our data agree with those of FORTH et al.[3,4], who carried out experiments in vitro on isolated small intestine segments of the rat and guinea-pig and in vivo on small intestine loops of the rat. They found the following values of ouabain intestinal absorption: 1. After 2 h, 3.8% in isolated small intestine segments of the rat and 15% in small intestine segments of the guinea-pig. 2. After 20 min 10% in small intestine loops of the rat in vivo. In all these experiments, FORTH et al. found that the quantity of ouabain absorbed was closely proportionate to the dose administered. In conclusion, both the results obtained by FORTH et al. and by us clearly demonstrate that the absorption process of ouabain administered orally to guinea-pigs is quite linear.

Riassunto. Nella cavia l'assorbimento intestinale della ouabaina-^{3}H è risultato del 3,6%, del 6–7% e del 9,5% rispettivamente dopo 1, 5 e 15 ore dalla somministrazione orale in un intervallo di dosi tra 250 e 1000 μg/kg. I rapporti tra le quantità di ouabaina-^{3}H somministrate oralmente e le quote di farmaco assorbite attraverso la parete intestinale sono risultati lineari.

A. MARZO, L. MERLO,
V. NOSEDA and G. V. MARCHETTI

Laboratorio di Biochimica della Simes S.p.A., Via Bellerio 41, I-2016 1 Milano (Italy), and Istituto di Cardiologia Sperimentale Simes S.p.A., Milano (Italy), 10 June 1970.

[9] G. VOGEL and I. BAUMANN, Arzneimittel-Forsch. *19*, 657 (1969).

Toxicity of Streptomycin and Terramycin, and Influence on Growth and Developmental Time of *Drosophila melanogaster*

The influence of the antibiotics streptomycin and terramycin on growth and developmental time of *Drosophila* have been studied. Freshly hatched larvae[1] were placed in polystyrol beakers containing 33 ml of rearing medium with different concentrations of the antibiotics. The rearing medium was a modified medium C of SANG[2] in which the caseine was replaced by 3 times its weight of defatted, desalted powdered milk, and by adding 12% of a water extract of dry brewer's yeast. Solutions of the antibiotics were added to the medium after it had cooled down to about 50 °C and were thoroughly mixed with the medium before it solidified.

Drosophila eggs were washed out of ordinary rearing bottles containing a standard corn agar medium with fresh baker's yeast on which 100 to 200 pairs of 3-day-old flies had been allowed to lay eggs for 4 h. The water was passed through a fine meshed metal sieve which retained fragments of the medium but allowed the passage of the eggs. These were collected on a piece of fine meshed gauze, washed thoroughly with water, desinfected for 10 min in 70% ethanol, rinsed with distilled water, and incubated on wet filter paper at 25 °C. The freshly hatched larvae were transfered on the rearing medium by means of a fine brush. Each beaker received 50 larvae. Two replicates were prepared for each concentration of the antibiotics. The rearings took place in a dark room at 25 °C and 50% relative humidity. In the control media without antibiotics pupation began on the sixth day. The newly formed pupae and the eclosed flies were counted each day. The mortality data were subjected to probit

[1] Thanks are expressed to Prof. WÜRGLER of the Zoological Institute for supplying us with fresh *Drosophila* eggs.
[2] I. K. SANG, J. exp. Biol. *33*, 45 (1956).

analysis after FINNEY[3] using a computer program[4]. The length of the puparia was measured under the stereomicroscope and used as an index of growth.

Streptomycin was tested up to the concentration of 1.5% w/v. Concentrations up to 0.2% were seemingly not toxic since neither the developmental time nor the size of the puparia were affected. Even the concentration of 0.4% did not cause mortality, though toxicity became apparent, the size of the puparia being significantly reduced (Table I) and larval development slowed down (Figure 1). These growth effects became much more pronounced at higher concentrations. The length of the puparia was reduced by 29.3% at the concentration of 1.4% streptomycin and the mean time of larval development was doubled at this concentration. Prolongation of the pupal developmental time has never been observed. Above the concentration of 0.4% larval and pupal mortality increased with a slope of 0.98 ± 0.29 until the critical concentration of 0.6% was reached. After that mortality increased very strongly, giving a straight dose-mortality-curve with a slope of 7.28 ± 0.7 (Figure 2A). Most insects died as larvae. Pupal mortality never surpassed 10% of the total number of test insects and thus had only some significance below the LC_{50}. The proportion of dead pupae to all dead insects amounted to 35% at the LC_{20} and to 20% at the LC_{50}. The differences between the curves for larval and total mortality were not significant. Therefore only the latter has been drawn in Figure 2. The LC_{50} values were 1.12% for larval and 1.02% for total mortality (Table II). Teratogenic effects, i.e. phenotypical modifications of the eclosed flies were never observed. The longevity of the adults has not been studied.

Streptomycin has several active groups and may therefore act at different cell levels[5–11]. One of the most important mechanism seems to be misreading in protein synthesis[12]. Our results indicate that *Drosophila* larvae are not very sensitive to orally taken streptomycin. Since the antibiotic has not been injected it is not known whether or not this is also true for the tissues and cells. The low toxicity of ingested streptomycin might simply reflect weak resorption of the antibiotic in the gut. However, there is also evidence suggesting that resorbed streptomycin is continuously detoxified in the larval system of *Drosophila*. Otherwise a higher percentage of insects dying as pupae would be expected. The low pupal mortality indicates that streptomycin does not accumulate in the individuals as, for instance, the 'exotoxin' of *Bacillus thuringiensis*, where the dose-mortality-curves for larval and total mortality do not strongly overlap[13]. Toxic effects are therefore only possible if the antibiotic is ingested continuously.

[3] D. J. FINNEY, *Probit Analysis* (University Press, Cambridge 1952).
[4] R. J. DAUM and W. KILLCREAS, Bull. ent. Soc. Am. *12*, 365 (1966).
[5] D. S. FEINGOLD, Biochim. biophys. Acta *55*, 787 (1962).
[6] R. HANCOCK, J. gen. Microbiol. *28*, 493 (1962).
[7] C. E. FREDA and S. S. COHEN, J. Bact. *92*, 1680 (1966).
[8] T. M. STAEHELIN, J. molec. Biol. *19*, 207 (1966).
[9] H. KAJI, Biochim. biophys. Acta *134*, 134 (1966).
[10] H. SCHMIDT, Pharmacie *23*, 161 (1968).
[11] A. M. WALTER and L. HEILMEYER, *Antibiotica Fibel* (Thieme Verlag, Stuttgart 1965).
[12] B. J. HARRISON, Nature, Lond. *213*, 990 (1967).
[13] J. M. PERRON and G. BENZ, J. Invertebrate Path. *10*, 379 (1968).

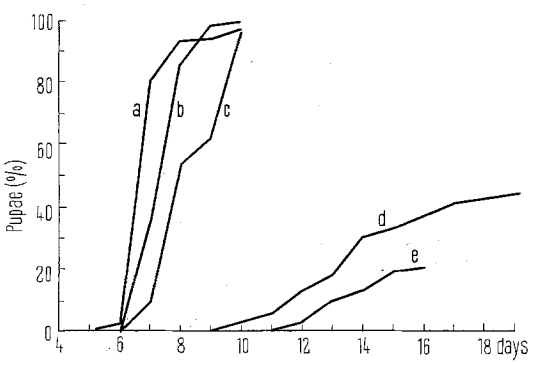

Fig. 1. Time to puparium formation in days (abscissa) with different concentrations of streptomycin in larval diet: a, 0.05%; b, 0.4%; c, 0.8%; d, 1%; e, 1.5%.

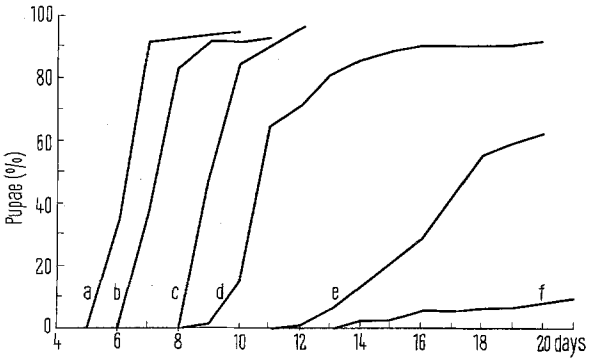

Fig. 3. Time to puparium formation in days (abscissa) with different concentrations of terramycin in larval diet: a, 0; b, 0.005%; c, 0.025%; d, 0.05%; e, 0.1%; f, 0.2%.

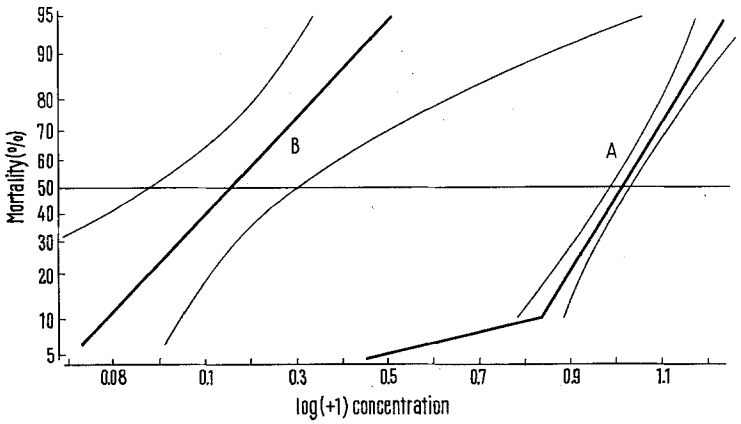

Fig. 2. Calculated probit regression lines with lower and upper fiducial limits (5% probability level) for total mortality caused by A) streptomycin and B) terramycin.

Table I. Length of puparia with different concentrations of streptomycin in percentage of larval diet (40 puparia per concentration). Values of mortality are approximative

Streptomycin concentration	Mortality caused (%)	Length of puparia $\bar{x}$ $s_{\bar{x}}$	Significance[a]
0	0	2.99 ± 0.03	a
0.2	0	2.99 ± 0.03	a
0.4	0	2.83 ± 0.03	
0.6	10	2.68 ± 0.03	
0.8	30	2.35 ± 0.04	b
1.0	50	2.37 ± 0.04	b
1.2	70	2.37 ± 0.06	b
1.4	85	2.12 ± 0.05	

[a] Values with same letters are not different at the 5% level.

Table II. Calculated concentrations of streptomycin (in %) with lower (LFL) and upper (UFL) fiducial limits for larval and total mortality

Mortality (%)	Larval mortality			Total mortality		
	Concentration	LFL	UFL	Concentration	LFL	UFL
10	0.735	0.638	0.806	0.679	0.608	0.679
30	0.942	0.965	1.090	0.863	0.805	0.926
50	*1.118*	1.056	1.118	*1.019*	0.964	1.077
70	1.325	1.244	1.452	1.203	1.130	1.280
90	1.701	1.539	1.987	1.529	1.384	1.688
b	7.035 ±	0.734		7.280 ±	0.698	

b, slope of regression line.

Table III. Same as Table II, for terramycin

Mortality (%)	Larval mortality			Total mortality		
	Concentration	LFL	UFL	Concentration	LFL	UFL
10	0.071	0.043	0.090	0.062	0.021	0.088
30	0.098	0.072	0.118	0.089	0.047	0.120
50	*0.122*	0.099	0.148	*0.115*	0.077	0.161
70	0.153	0.127	0.197	0.148	0.109	0.248
90	0.209	0.169	0.322	0.214	0.154	0.549
b	5.46 ±	0.83		4.73 ±	1.01	

The disconnected change of slope of the dose-mortality-curve above the critical concentration of 0.6% indicates an abrupt change in the intoxication system. The nature of this change cannot be deduced from our results. It seems that, below the critical concentration, relatively few molecules of streptomycin reach vital centers of the cells, whereas disproportionately more molecules reach such sites above that concentration. Since the proportion of insects killed as pupae is always low, this change is probably not due to a breakdown of the detoxifying system, but it might reflect a change in the permeability of the cell membranes as demonstrated in *Escherichia coli*[5,11]. However, a change in the resorption system cannot be excluded.

Terramycin proved to be far more toxic for *Drosophila* than streptomycin (Figure 2B and Table III). No mortality occurred up to a concentration of 0.025%, but mortality became significant when the medium contained 0.06% and more terramycin. The LC_{50} of terramycin is 0.115% (fiducial limits at 5% level = 0.08 and 0.16), i.e. smaller than that of streptomycin by a factor of 5.9, if the 2 values are expressed as molar concentrations. However, the dose-mortality-curve of terramycin with the slope of 4.74 ± 1.01 is less steep than that of streptomycin. Similarly to the action of the latter, but even more pronounced, terramycin kills most insects as larvae. At the LC_{50} only 10% of the dead insects were pupae and none at the LC_{80}.

The higher toxicity of terramycin is also demonstrated by the fact that sublethal concentrations prolongate larval development considerably, e.g. by 1 day with 50 ppm, 3 days with 250 ppm, and more than 4 days with 500 ppm (Figure 3). At the concentration of 0.1%, corresponding to an LC_{40}, the mean time of larval development was 2.7–3 times longer than that of the controls. However, prolongation of pupal development has never been observed and the hatching flies were apparently normal.

The high toxicity of terramycin in *Drosophila* seems to indicate that this antibiotic is better resorbed in the gut and diffuses more quickly to the tissues and into the cells than streptomycin, as found in man[14]. The short half-life of terramycin[11], on the other hand, is reflected by its almost exclusively larvicidal action.

It may be interesting to note that, at a concentration of terramycin causing 100% mortality, the larvae may survive up to 24 days as L1 or tiny L2. They do not grow and eventually die. However, this phenomenon is not specific of terramycin. In our laboratory it was also observed in larvae put on a salt-free medium. Such larvae did not lose the potency of growth; they grew perfectly well as soon as a salt mixture containing K, Na, Mg, carbonate, phosphate and sulfate was added to the medium. Whether or not the tiny larvae from a terramycin medium would also retake normal growth when put on a normal medium has not been tested. However, the observations indicate that even a highly lethal dose of terramycin does not interfere specifically with the maintenance metabolism of *Drosophila* larvae, but rather suppresses the synthesis of substances necessary for growth.

It is known that tetracyclins interfere with the synthesis of ribosomes[15,16]. Suppression of the synthesis of ribosomes by 5-fluorouracil also completely inhibits the growth of *Drosophila* larvae[17]. Thus the toxic action of terramycin might base on this mechanism rather than on its potency to form chelates with ions of heavy metals[14].

Zusammenfassung. Die toxische Wirkung von oral an Larven von *Drosophila melanogaster* verabreichtem Terramycin ist bedeutend grösser als jene von Streptomycin. Bei beiden Antibiotica lässt die geringe Puppenmortalität auf einen wirksamen Entgiftungsmechanismus schliessen.

E. Graf and G. Benz

Entomologisches Institut der Eidgenössischen Technischen Hochschule, CH-8006 Zürich (Switzerland), 23 July 1970.

[14] A. Kraushaar, in *Die Antibiotica* (Verlag Hans Carl, Nürnberg 1962), p. 502.
[15] T. J. Franklin, Biochem. J. *90*, 624 (1964).
[16] I. A. Holmes and D. G. Wild, Biochem. J. *97*, 277 (1965).
[17] J. B. Jenkins and J. R. Simmons, Drosoph. Inf. Serv. *43*, 150 (1968).