or i.c. RNA, hydrolyzed RNA or saline with respect to either acquisition or latency.

However, these experiments with yeast RNA would shed no light on the possible involvement of RNA synthesis or DNA derepression on learning as some of the more sophisticated theories imply[9]. Since results of experiments with several of the antibiotic inhibitors of RNA and DNA synthesis are somewhat controversial[9], an attempt was made to influence the learning ability of rats through i.c. injected TU[10]. This antibiotic is an inhibitor of DNA, RNA, and protein synthesis through its incorporation in place of adenosine[11]. In several experiments non-toxic amounts of i.c. TU did not affect the learning of a shuttle-box avoidance situation. An example of this is included in the table of results, along with those obtained with various histone fractions. Lysine-rich histone fraction from calf cerebellum was injected i.c. to assess if such administration would influence, possibly through binding to DNA, the synthesis of m-RNA. There is evidence from in vitro experiments that histones are inhibitors of RNA synthesis. It was assumed that through a mechanism similar to that in vitro lysine-rich histone may interfere with the in vivo synthesis of protein necessary for learning with the resultant changes in acquisition and/or latency.

I.c. injection of arginine-rich histones and bovine serum albumin served as controls in 2 groups of rats. Prolonged administration of either histone fractions or bovine serum albumin did not produce any toxic side effects, nor did they influence the performance of the animals. These results, based on careful control of experimental conditions, confirm those who reject the notion that administration of RNA has any relevance to learning or memory[12].

Zusammenfassung. RNA aus Hefe, RNA-Hydrolysat, Tubercidin und mit Lysin angereichertes Histon, i.c. oder i.p. injiziert, hatten keine Wirkung auf die Lernfähigkeit von Ratten. Intracerebral injiziertes ^{14}C-RNA verbreitete sich schnell über das ganze Gehirn und verschwand relativ schnell wieder. Nach i.p. Injection von ^{14}C-RNA konnte jedoch ein geringer Betrag von ^{14}C im cerebralen RNA gefunden werden.

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[10] The daily intracerebral injection of TU must not exceed 2.5 µg of the antibiotic. Above that level TU produces toxic side effects which interfere with the behavioral testing. However, even at this level the period of injection should not exceed 7 days since the total cumulative effect of over 17 µg TU will decrease survival to 60% with the avoidance response of survivors dropping from an average of 67% to about 30%. With 40 µg total TU injected the survival drops to 10% with an avoidance response of about 7%. (These poor performance responses must be attributed to illness rather than a specific effect on learning ability.)

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Antagonism of Terramycin on Action of *Bacillus thuringiensis* 'Exotoxin' in *Drosophila melanogaster*

The heat-stable 'exotoxin' (ET) produced by certain serotypes of *Bacillus thuringiensis* has the chemical nature of a complicated nucleotide, which in addition to adenine, ribose, and phosphate[1-3] contains the unusual constituents glucose and allomucic acid[4-6]. The substance has the molecular weight of 755 and has been named thuringiensis A. The corresponding γ-lactone is nontoxic and is called thuringiensis B[6]. Because of the chemical nature of ET, the hypothesis had been forwarded that it might act as an antimetabolite of the nucleic acid metabolism[2]. This hypothesis has since been verified to some extent by Czech authors who found reduced incorporation of orotic acid and cytidine into the liver RNA of mice[7] and inhibition of DNA-dependent RNA polymerase in *Escherichia coli*[8]. The following report of an antagonism of terramycin and ET in *Drosophila* may throw further light on the mechanism of action of ET in an insect.

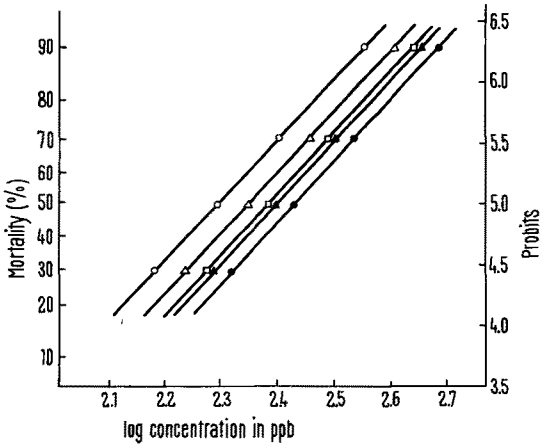
The toxicity of ET in *Drosophila* reared on a yeast free medium and the antagonistic effect of yeast and certain yeast extracts has been described earlier[9,10]. For toxicological data on terramycin and information on the rearing and assay method used in the present work, the reader should consult another paper[11]. Terramycin allows complete survival of *Drosophila* on a medium containing up to 250 ppm of the antibiotic, whereas concentrations above 600 ppm cause significant mortality (LC_{50} = 0.115%). In the present study the concentrations of terramycin have therefore been restricted to 200 ppm or less. However, even sublethal concentrations of 100 to 250 ppm of terramycin reduce the rate of larval growth and delay the time of pupation by 16 to 50%. The influence of terramycin on the toxic action of ET was investigated in combinations where different concentrations of both the antibiotic and ET were added to the rearing medium of the larvae. The ET preparation used was a prepurified[2] culture medium of *B. thuringiensis var. thuringiensis*. Homologization with a sample of pure ET (kindly furnished by Dr. R. P. M. BOND of the Milstead Laboratory, Sittingbourne, England) indicated that our preparation had an ET concentration of 100 ppm. Total mortality was recorded for ET alone and the combinations. The data were subjected to probit analysis, using 2 computer programs[12].

Except for very low doses, ET alone gave straight dose-mortality curves with a slope of 4.5 to 5.0, i.e. similar to lethal doses of terramycin alone[11]. Correspondingly the dose-mortality curves of the combinations of ET with terramycin had the same slope (Figure). However, the LC_{50} values for the combinations are higher than the LC_{50} of ET alone, indicating an antagonistic action of the antibiotic.

The antagonistic effect of different concentrations of terramycin can be determined if the factor of the parallel displacement of the curves, i.e. the potency (P) is calculated[12], P of ET alone having the value of 1. The LC_{50}

values and the respective P values with their upper and lower fiducial limits are computed in the Table. Since all curves with terramycin indicate higher LC_{50} values than ET alone, only the lower fiducial limits are important. Values larger than 1 indicate that the displacement of the corresponding curves are significant at the 5% level. The Table shows significant antagonism of terramycin for all concentrations of 15 to 200 ppm. The curves of the lower concentrations of the antibiotic show the same tendency, but the displacement is statistically not significant.

The highest antagonistic action was found with the terramycin concentration of 50 ppm. The results suggest that an optimal concentration of terramycin for ET antagonism exists. It is not known whether or not this concentration is exactly 50 ppm, because no concentration between 50 and 200 ppm has been tested. However, since 50 ppm of terramycin prolong the time of larval development by one day[11] and represent the lowest dose causing a slight but significant reduction of the size of puparia, 50 ppm might well be the optimum concentration of terramycin for ET antagonism. With higher concentrations of the antibiotic, the negative effects may neutralize the antagonistic action.



Influence of terramycin on lethal concentrations of 'exotoxin'. Concentrations of terramycin in ppm: ○ = 0; △ = 9; ▲ = 15; ● = 50; □ = 200.

LC_{50} values for ET combined with different concentrations of terramycin and factor of antagonism (potency) with lower (LFL) and upper fiducial limits (UFL). LFL values greater than 1 indicate significant antagonism at the 5% level

Terramycin ppm	ET concentration LC_{50} in ppm	Potency	LFL	UFL
0	196.77	1.000	–	–
5.4	221.68	1.127	0.975	1.301
9	222.97	1.132	0.979	1.307
15	252.72	1.284	1.108	1.488
50	270.73	1.375	1.187	1.594
200	250.63	1.274	1.103	1.470

The fact that the dose-mortality curves for ET alone and terramycin alone have the same slope might indicate that the two substances hit the same or a closely related target within the *Drosophila* organism, though almost 10^4 times more molecules of terramycin are needed to produce the same effect. It is known that terramycin interferes with the synthesis of ribosomes[13,14] and that ET suppresses the synthesis of RNA in mice[7] and bacteria[8]. Therefore a synergistic rather than an antagonistic relation of the two substances would be expected. However, since no lethal concentration of terramycin has been combined with ET, the question of synergism remains open. Our results indicate ET antagonism only for non-lethal concentrations of terramycin with an optimum near 50 ppm. With this concentration a larva consuming 2 mg of the medium ingests about 128×10^{12} molecules of the antibiotic and has to ingest 0.43×10^{12} molecules of ET for an LD_{50}. Thus antagonism is maximal when about 300 molecules of terramycin are ingested with each molecule of ET. Without the antibiotic 0.31×10^{12} molecules of ET are sufficient for an LD_{50}. The figures show that 128×10^{12} molecules of terramycin antagonize the lethal action of 0.12×10^{12} molecules of ET, i.e. about 1000 molecules of the antibiotic are needed to antagonize the action of one molecule of ET.

On first sight this high proportion of terramycin to ET seems not indicative of a direct competitive antagonism. However, terramycin at lethal concentrations kills almost exclusively the feeding larvae, death of pupae being rare[11]. This indicates that the antibiotic is soon detoxified in *Drosophila* and that it is not firmly bound to its substrate. The high proportion of terramycin thus may reflect only the short half-life of the substance, as does the relatively high LC_{50} mentioned above.

ET on the other hand may accumulate in the organism. At low concentrations it produces a high rate of pupal mortality in *Drosophila*[10] and only pupal mortality in *Musca*[15]. These facts indicate that ET is not easily tetoxified in Diptera and that it is firmly bound to its substrate.

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The facts are compatible with the hypothesis that ET and terramycin compete for the same or a metabolically closely related substrate. Relatively low concentrations of terramycin may antagonize the action of ET because terramycin is not firmly bound to the substrate and may therefore become detoxified later on. An additional point in favour of this hypothesis is the fact that mutual antagonism occurs. A concentration of 200 ppm of terramycin alone prolongates the time of larval development by 3 days, but has not this effect when combined with ET. In the combinations the time needed to complete larval development is shorter and corresponds to that with ET alone.

Zusammenfassung. Subletale Konzentrationen von Terramycin hemmen in *Drosophila* teilweise die toxische Wirkung des hitzestabilen Exotoxins von *Bacillus thuringiensis*, wobei die Steilheit der Dosis-Mortalitätskurven nicht verändert wird. Es wird die Hypothese begründet, dass es sich um eine kompetitive Hemmung des Exotoxins durch das Antibioticum handeln könnte.

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Ultrastructural Aspects of the Pancreatic Islets in Carps of Spontaneous Diabetes Mellitus

It has long been known that, during the course of feeding, culture carps frequently show the atrophy of their dorsal muscles which is a widely known symptom called Sekoke in Japan. A series of pathological and biochemical studies on carps with such symptoms[1-3] have disclosed that they are cases of spontaneous diabetes mellitus. According to the light microscopic observations on the pancreatic islets of the diabetic fish, the insular B cells are found to undergo degranulation and marked glycogen deposition, and there are more clear islet cells than in the insular tissues of normal fish[1].

The aim of the present study is to observe the ultrastructural aspects of the pancreatic islets in the diabetic and normal carps and to interpret the nature of the spontaneous diabetes in terms of the modified secretory activity of the islet cells.

A total of 20 diabetic and 50 normal carps of both sexes, ranging in age from 6 months to 1 year, were used. Principal islets from these donor animals were fixed at 0 °C in either modified Dalton's fixative[4] or phosphate buffered (pH 7.4) 2% osmium tetroxide, dehydrated and embedded in Epon 812. Some of the tissues were fixed at the same temperature in phosphate buffered (pH 7.4) 2.5% glutaraldehyde, postfixed in similarly buffered 2% osmium tetroxide (pH 7.4) and processed as above. Thin sections of the islet tissues were cut on a Porter-Blum microtome, doubly stained with methanolic uranyl acetate and aqueous lead citrate and examined with an electron microscope (Hitachi Hu 11A). The pancreatic islets of normal carps consist of 4 types of glandular cells, A, B, clear and D cells (Figure 1). The A and B cells are easily identified by the criteria previously postulated[5] in the same species of the teleost. The A cell is provided with an indented nucleus and with a relatively electron dense cytoplasm containing 200–270 nm sized round secretory granules of high electron opacity. In contrast to this, the B cell is recognized by its 200–400 nm sized secretory granules which have polymorphous (crystalloid, homogeneous or finely particulate) cores. While Titlbach[5] previously regarded islet cells other than A and B cell types as being only one cell type, the D cell, we can differentiate here, with certainty, a clear cell from the D cell in addition to A and B cells. The cytoplasm of such clear cells fixed in modified Dalton's or buffered osmium tetroxide solutions contains varying numbers of vesicles with a diameter ranging from 150 to 500 nm, moderate numbers of cisternae of the granular endoplasmic reticulum and a few secretory granules. In the clear cells, occasional granules are found to encase crystalloid cores characteristic of B cells. If the islet tissues are subjected to double fixation with glutaraldehyde and osmium tetroxide, vesicles within the clear cell cytoplasm acquire a high electron opacity. Furthermore, contiguous thick (1 μm) sections stained with aldehyde fuchsin reveal that the content of the clear cell vesicles is reactive for this reagent. These cytological properties of the clear cells strongly suggest that the clear cell is a variety of the B cell. The D cell is a rather dark cell type containing 150–220 nm sized secretory granules and a few slightly dilated cisternae of granular endoplasmic reticulum.

The pancreatic islets of diabetic (Sekoke) carps are characterized by the presence of appreciably more numerous clear cells than those in the analogous glands

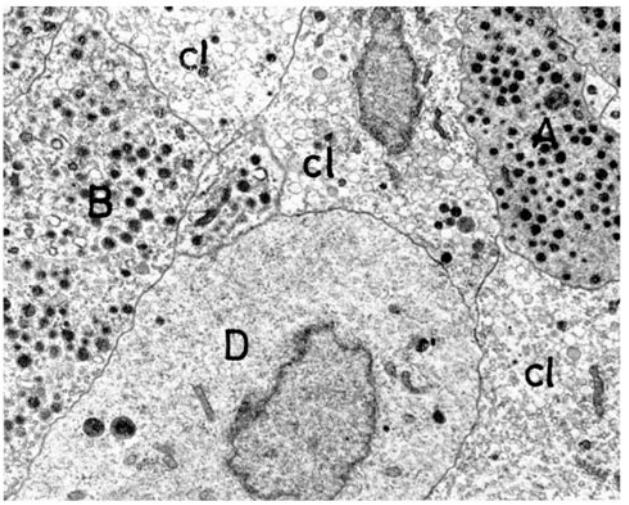

Fig. 1. Electron micrograph of part of the pancreatic islet in a normal carp. A cell (A), B cell (B), clear cell (cl), D cell (D). Modified Dalton's solution fixation. ×6000.

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