in Spuren nachweisbar waren. Bei einem Stamm von *T. cinnabarina* trat neben der Verbindung A noch eine zweite Hauptkomponente B auf. Bei der Kultivation dieses Stammes in der NL 2 (Glucose 10,0 g; Pharmamedia ®, Traders Protein Division, Fort Worth, USA 5,0 g; KH_2PO_4 2,0 g; $MgSO_4 \cdot 7H_2O$ 0,1 g; Aqua dest. ad 1000,0 ml) liess sich Verbindung B in 40prozentiger und Verbindung A in 10prozentiger Ausbeute isolieren. Die Umsetzung ist also auch hier abhängig von der Zusammensetzung des Fermentationsmediums.

Die Verbindung A konnte durch Schmp., UV-, IR- und Massenspektren sowie Vergleich mit einem nach[10] hergestellten synthetischen Präparat als 14-β-Hydroxycodeinon (II) identifiziert werden.

Das Produkt B erwies sich als 14-β-Hydroxycodeinon-N-oxid (IV). Besonders aufschlussreich war das Massenspektrum. Der Peak des Molekularions fand sich bei m/e 329. Die relative Intensität betrug 10%. Charakteristisch für aromatische N-Oxide ist das Auftreten von starken Peaks bei M-16 und M-17[11–13]. In unserem Fall lag der «base-peak» bei der MZ 313 (M-16). Das Fragment der MZ 312 (M-17) erschien mit 65% der Intensität des «base-peaks». Im 100-MHz-NMR-Spektrum (aufgenommen in $CDCl_3$) war die Lage der aromatischen Protonen und der Doppelbindungsprotonen am C-7 und C-8 in beiden Verbindungen (II und IV) identisch. Das gleiche trifft für die Protonen der OCH_3-Gruppe zu. Das Drei-Protonen-Singulett bei 7,67 ppm (τ-Skala) ($N\text{-}CH_3$) von II war bei IV zu niederer Feldstärke verschoben (typisch für aromatische N-Oxide).

Das Produkt B zeigte ferner die gleichen Eigenschaften wie eine nach der Vorschrift von SPEYER und STARRE[14] hergestellte Vergleichsprobe von 14-β-Hydroxycodeinon-N-Oxid.

Ebenso wie bei den japanischen Autoren trat auch bei unseren Versuchen als erstes Umwandlungsprodukt von (I) 14-β-Hydroxycodeinon (II) auf. Bei den meisten der von uns geprüften Stämme war II das Endprodukt. *T. cinnabarina* war in der Lage, II zu IV zu oxydieren. Im Gegensatz zu den japanischen Stämmen liess sich das Reduktionsprodukt von II, das 14-β-Hydroxycodein, nur gelegentlich in Spuren nachweisen, aber nicht kristallin fassen.

Die Bildung von N-Oxiden bei der mikrobiellen Umwandlung von Naturstoffen ist unseres Wissens bisher noch nicht beschrieben worden.

Summary. Thebaine was transformed into 2 products by fermentation with a strain of *Trametes cinnabarina*. The 2 compounds were isolated and identified as 14-β-hydroxycodeinone and 14-β-hydroxycodeinone-N-oxid.

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Oral and Parenteral Toxicity of *Bacillus thuringiensis* 'Exotoxin', and its Inactivation in Larvae of *Galleria mellonella*

MCCONNEL and RICHARDS[1] found evidence for the presence of the so-called 'exotoxin' of *Bacillus thuringiensis* (ET) by injecting autoclaved culture medium of this bacillus into the hemocoel of larvae of *Galleria*. They claimed that the active substance was not toxic for the same insect when given orally. In other insects, however, it has been found that the ET is active when ingested[2,3], and recently it was demonstrated that *Galleria* too was susceptible to the orally applied substance[4,5]. Thus *Galleria* would differ from many other insects only in so far as relatively high concentrations of ET are needed for oral intoxication. However, no exact comparisons have been published as yet.

Our work with *Galleria* was undertaken in order to clarify the quantitative relations between oral and parenteral toxicity. At the same time we wanted to know why larvae of *Galleria* were less susceptible to the orally applied substance. Two hypotheses had to be tested: (1) The toxic substance might be absorbed so slowly by the gut epithelium that only very small quantities could reach the hemocoel, whereas the majority of it would be eliminated with the feces. (2) The toxic substance could be inactivated in the intestinal tract by enzymatic degradation and/or formation of an inactive complex.

Thirty-day-old larvae (40–60 mg) were injected via an abdominal proleg with 0.5 µl of different concentrations of ET, prepurified by differential precipitation[6]. Other larvae of the same age and weight were put singly in glass tubes, each containing 50 mg of rearing medium with different amounts of ET. After 6 days, when the larvae had fed all the medium, new untreated food was given. Thus the amount of ET fed per larva was known. In both types of experiments the mortality was recorded. The data were corrected for spontaneous mortality (8–10%) and subjected to probit analysis, using 2 computer programms[7]. In this paper doses are expressed as µl of our standard solution of prepurified ET.

Figure 1 shows the 2 dosis-mortality curves with their 95% fiducial limits for larvae which were injected and fed ET. The 2 respective LD_{50} values and the slopes (b) of the curves are 0.0078 µl/larva ($b = 2.03 \pm 0.44$), and

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1.93 µl/larva (b = 1.44 ± 0.37). Since these slopes are not significantly different the curves can be regarded as parallel, having an intermediate slope b = 1.73 ± 0.27 and LD_{50} values of 0.0067 and 1.87 µl/larva.

The results demonstrate that the ingested ET is 247–278 times less toxic than the injected ET. The similarity between the slopes of the curves suggests on the other hand that the mode of action of injected and ingested ET is probably much the same.

Similar experiments in which a better purified ET-preparation with less than 0.3% dry substance was injected gave an LD_{50} of 0.0262 µl/larva. Expressed in units of weight the LD_{50} is less than 0.078 µg/larva or 1.56 µg/g body weight. The latter figure compares very favourably with DDT and Pyrethrum which, injected into larvae of *Galleria*, have LD_{50} values of about 250 µg/g[8,9]. Thus the toxicity of injected ET must be more than 160 times higher than either one of these insecticides. However, DDT has the same toxicity whether it is injected or ingested[8], whereas Pyrethrum is practically non-toxic when fed to larvae of *Pieris* in leaf sandwiches[10]. The facts indicate that both, Pyrethrum in *Pieris* and ET in *Galleria*, are either inactivated in the gut or not easily taken up from the gut contents into the hemocoel.

In order to find out whether or not ingested ET was excreted with the feces, isolated larvae were fed different amounts of ET in 50 mg/larva of a yeast-free *Galleria* medium[11]. When the larvae had eaten the whole portion of treated food, the frass pellets were collected, mixed well with yeast-free *Drosophila* medium and bioassayed with newly hatched larvae of *Drosophila*[12]. Control experiments in which feces of untreated *Galleria* were added to *Drosophila* medium showed that these feces were not toxic for *Drosophila*. In other control experiments different amounts of ET were added to *Drosophila* medium containing such feces. The mortality values obtained fitted well with a dosis-mortality curve for *Drosophila* established previously on the same medium without *Galleria* feces[13]. Thus the addition of normal *Galleria* feces does not interfere in any way with ET toxicity.

Figure 2 shows the results of bioassays with the feces of *Galleria* larvae fed with 0.313, 0.625 and 1.25 µl of ET. It is evident that the feces of all larvae contained a constant amount of some toxic principle, independent of the dose of ET fed to the larvae. If the toxic substance excreted by the larvae is unchanged ET, which has not been investigated, it corresponds to about 0.262 µl/larva.

The results indicate that of the ingested ET about 0.051 µl at the lower, 0.363 µl at the middle, and 0.988 µl at the highest dose did not reappear in the feces, i.e. amounts which are linearly correlated to the doses fed. If this correlation is true (the experiment has not been repeated) it would mean that an oral dose of about 0.26 µl/larva will be completely eliminated with the feces, whereas all higher amounts of ET are either destroyed or kept back in the body of the *Galleria* larva. We can be quite sure that only a fraction of the non-excreted ET enters the hemocoel, since we found only 12% mortality for the lowest and 50% for the highest dose. If the retained ET entered the hemocoel, about 95% mortality would be expected with the lowest dose, and more

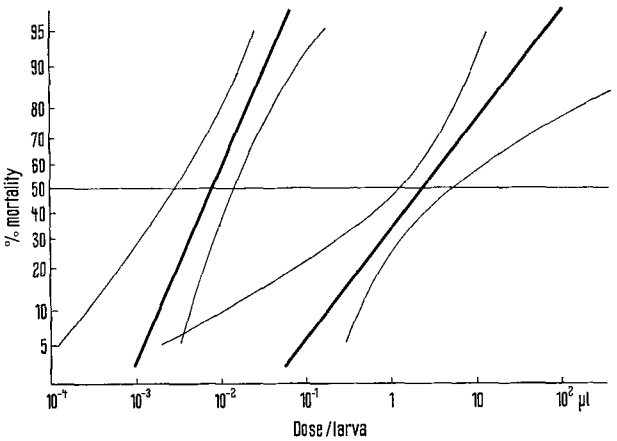

Fig. 1. Dosis-mortality curves with 95% fiducial limits for injected (left curve) and ingested ET.

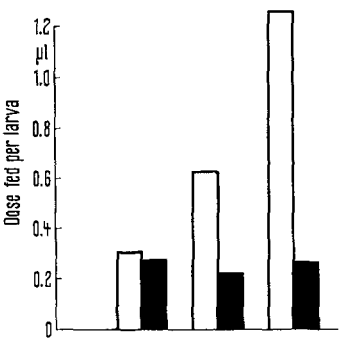

Fig. 2. Doses of ET fed to larvae (white columns) and toxic substance, presumably unchanged ET, recovered in feces (black columns).

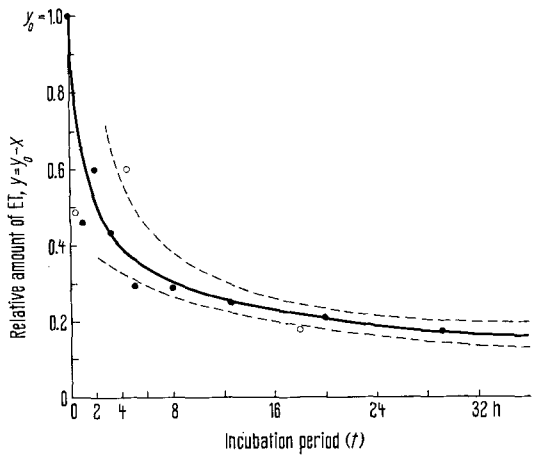

Fig. 3. Relative amount of ET (y) recovered from larvae microfed with 2.5 µl of ET ($y_0 = 1$) and incubated for different lengths of time (t) at 31 °C (full signs) and 22 °C (open signs). The curve fitted to the values follows the function $y = y_0 - e^{-k \cdot t^{-0.5}}$. The broken lines indicate the confidence limits of the *Drosophila* test (see text) and do not take into account the variation in the *Galleria* larvae. The signs which lie not far outside these limits may therefore be considered to be within the true fiducial limits of the drawn curve.

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with the higher doses. 12–50% mortality correspond to injected doses of 0.0018–0.0095 µl/larva. It is therefore concluded that 96–99% of the retained ET is inactivated or, although rather unlikely, stored in the gut.

The question was further investigated in a third set of experiments. Larvae of *Galleria* were starved for 24 h and then microfed an LD_{50} of ET standard solution (2.5 µl/larva). The treated larvae were killed either directly after microfeeding by putting them into a deep freeze box at −22 °C, or after incubation for different lengths of time in empty glass tubes at 31 °C in one experiment, and at room temperature of about 22 °C in another experiment. Since the treated larvae were not fed, they produced no feces so that no ET could be lost with the frass. The dead larvae were autoclaved, and homogenized in groups of 2 larvae per 4 ml of water. The homogenates were added to *Drosophila* medium and bioassayed with *Drosophila* larvae. The dose of ET contained in 2 *Galleria* larvae, killed immediately after injection, produced 99.9% mortality in the *Drosophila* test. Homogenates of larvae that had been kept alive for varying lengths of time after microfeeding produced less mortality. With the aid of the *Drosophila* dosis-mortality curve mentioned above, the mortality data were transformed into data of 'ET-concentration in medium', indicating the amount of ET per larva present in the homogenates.

Figure 3 shows the results of such an experiment. It demonstrates that within about 2 h half the toxicity of the ET is lost. Later on the process of detoxication slows down. If y_o is the dose of ET microfed and y the amount of ET still present after the incubation period t, the curve which best fits the results follows the function

$$y = y_o - e^{-k \cdot t^{-0.5}}$$

with the constant $k = 1.05$, calculated from the experimental values $t = 20$ h, $y = 0.21$ and y_o taken as 1. Thus the process of detoxication follows the function $x = y_o \cdot e^{-k \cdot t^{-0.5}}$ indicating that it is very probably achieved by a relatively complex enzymatic process. Since the results fit nearly as well on a curve of the function $y = y_o\,(1 + 2\,y_o{}^2 k\,t)^{-0.5}$, in which k is given the value of 0.6, it might be a third order reaction.

Incubation of the microfed larvae at 22 and 31 °C produced much the same results, indicating that the speed of the process is relatively temperature-independent. This is rather unusual for an enzymatic reaction. It is therefore probable that 2 or more enzymes with different temperature optima are involved in the detoxication process.

Zusammenfassung. Es wird gezeigt, dass Larven der grossen Wachsmotte durch oral appliziertes «Exotoxin» von *Bacillus thuringiensis* vergiftet werden, die LD_{50} aber ca. 250mal grösser ist als bei parenteraler Applikation. Oral verabreichtes «Exotoxin» wird zu einem grossen Teil im Raupendarm inaktiviert. Es konnte eine mathematische Beziehung zwischen der Inkubationszeit und der Inaktivierung des «Exotoxins» gefunden werden, die für einen enzymatischen Abbau des «Exotoxins» spricht.

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Antiviral Activity of Certain Substituted Purine and Pyrimidine Nucleosides

Certain derivatives of purine and pyrimidine nucleosides have marked antiviral activity, and of these 5-iodo-2′-deoxyuridine (IUdR, 'Idoxuridine') is in clinical use for treatment of *Herpes simplex* keratitis in man (references cited in reference[1]). The present report describes the antiviral activity of certain nucleosides against *H. simplex* virus in African green monkey kidney cells (BSC_1)[2] in vitro.

The methods used for growth of the virus, preparation of media and evaluation of infectivity and cytotoxicity have been described previously[3]. The compounds investigated were synthesized in the laboratory of Prof. R. K. ROBINS[4]. The Figure shows the structures of the substituted purine nucleosides. Replacement of one of the amino hydrogens in the 5′-position of 5′-amino-5′-deoxyadenosine (I) by a methylsulfonyl group forms the 5′-methylsulfonylaminoadenosine (II). Replacement of the hydroxyl in the 2′-position of compound I with a hydrogen forms 5′-amino-2′,5′-dideoxyadenosine (III). The Figure shows also the basic structure of the substituted pyrimidine nucleosides 6-methyluridine (IV) and 6-methylcytidine (V). Removal of the 6-methyl group of compounds IV and V and attachment of a phenyl ring at the 5,6-position forms 1-β-D-ribofuranosyl-2,4-quinazolinedione (VI) and 4-amino-1-β-D-ribofuranoxyl-2-quinazolone (VII), respectively.

The cytotoxicity of the various compounds studied are shown in Table I. Among the substituted purine nucleosides, 5′-methylsulfonylaminoadenosine was the least toxic compound, requiring a concentration of 5 mM to produce occasional toxicity. With the exception of 6-methyluridine no toxic effect was observed at a concentration of 2.5 mM, however, at higher concentrations cytotoxicity was produced by all compounds studied. The cytotoxic effects observed were of 2 types, one the gradual loss of refractibility of the cells, and the other a rounding of the cells with or without vacuolization in the cytoplasm. The different cytotoxic effects may be a reflection of different sites of action on the host cells. No correlation is apparent between the structure of the compound and the type of cytotoxicity produced.

Table II shows the antiviral activity of compounds I–VII. It can be seen that substitution of a methylsulfonyl group in the 5′-position of the 5′-deoxyribonucleoside (II) resulted in increased antiviral activity. Replacement of the hydroxyl group in the 2′-position of compound I with hydrogen produced compound III which not only in-

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