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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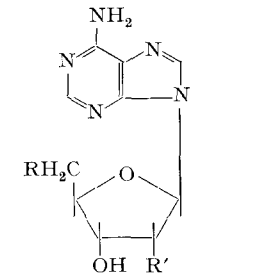
creased activity, but also increased toxicity (Table I). It may be possible to reduce the toxicity of compound III by substituting a methylsulfonyl group at the 5′-position. Conversion of the pyrimidine moiety of compounds IV and V to the corresponding quinazoline derivatives (VI and VII, respectively) did not alter significantly the antiviral activity, however, compound VI is less toxic to the host cells than the parent compound (IV).

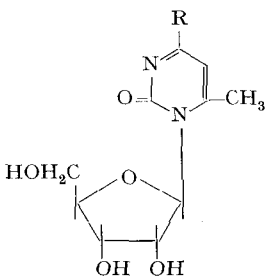
5′-Substituted nucleoside analogs were designed to be inhibitors of either nucleotide kinase or polynucleotide polymerase, and several adenosine derivatives were reported to have moderate antiviral activity[5]. The present study indicates that specific modification of 5′-amino-5′-deoxyadenosine increased its antiviral activity. The pyrimidine ribonucleosides were similarly moderately active as antiviral agents. Of significance is the moderately decreased toxicity of 6-methyluridine when converted to the corresponding quinazoline derivative.

Since the virus was adsorbed for 1 h at 37 °C prior to addition of the compounds, interference with virus adsorption and penetration as a site of inhibition of these compounds is minimized if not eliminated. The use of a high multiplicity of infection avoids a multiple cycle of replication of the virus, and indicates an effect on an intracellular process as being responsible for the observed antiviral activities.

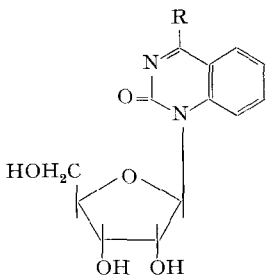
The biochemical basis for inhibition has not been investigated because of the modest antiviral activity of these



R = $-NH_2$, R′ = OH (I)
R = $-NHSO_2CH_3$, R′ = OH (II)
R = $-NH_2$, R′ = H (III)



R = OH (IV)
R = NH_2 (V)



R = OH (VI)
R = NH_2 (VII)

Structures of purine and pyrimidine nucleosides.

Table I. Cytotoxicity of certain substituted purine and pyrimidine nucleosides

Nucleoside	Reference No.	Concentration (m*M*)	Degree of toxicity
5′-Amino-5′-deoxyadenosine	I	0.5	0
		2.0	0
		4.0	++++
5′-Methylsulfonylamino-adenosine	II	0.5	0
		2.5	0
		5.0	±
5′-Amino-2′,5′-dideoxy-adenosine	III	0.5	0
		2.5	0
		5.0	++++
6-Methyluridine	IV	0.5	0
		1.0	0
		2.5	++
		5.0	++++
1-β-D-Ribofuranosyl-2-quinazolinedione	V	0.5	0
		1.0	0
		2.5	0
		3.5	++++
6-Methylcytidine	VI	0.5	0
		2.5	0
		5.0	++++
4-Amino-1-β-D-ribo-furanosyl-2-quinazolone	VII	0.5	0
		2.0	0
		2.5	0
		4.0	++++

Table II. Antiviral activity of certain substituted purine and pyrimidine nucleosides

Nucleoside	Reference No.	Highest concentration not toxic to host cells (m*M*)	Virus titer PFU/ml	% inhibition
No drug	–	–	1.3×10^6	–
5′-Amino-5′-deoxyadenosine	I	2.0	1.3×10^6	–
5′-Methylsulfonylamino-adenosine	II	2.5	3.5×10^5	73
5′-Amino-2′,5′-dideoxy-adenosine	III	2.5	2.9×10^5	78
6-Methyluridine	IV	1.0	4.6×10^5	65
1-β-D-ribofuranosyl-2,4-quinazolinedione	V	2.5	3.1×10^5	76
6-Methylcytidine	VI	2.5	6.3×10^5	52
4-Amino-1-β-D-ribo-furanosyl-2-quinazolone	VII	2.5	3.3×10^5	75

T. Neilson, R. Ruyle, R. L. Bugianesi, K. H. Boswell and T. Y. Shen, Abstr. Am. chem. Soc., p. 29 (Sept. 1967).

compounds, however, the value of certain modifications of structure in producing desired biological activity has been indicated. In view of the established marked antiviral activity of certain nucleosides it would appear that investigation of the potential antiviral activity of other nucleoside derivatives is warranted[6].

[6] This investigation was supported by grants from the National Cancer Institute of the U.S. Public Health Service (Nos. CA-05262 and CA-08109).

Zusammenfassung. Der virushemmende Effekt sowie die zytotoxische Aktivität gewisser Purine und Pyrimidin-Nukleoside gegen *Herpes-simplex*-Virus bzw. Kulturen von BSC-1-Zellen werden beschrieben.

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PRO EXPERIMENTIS

A Quantitative Evaluation of the Non-Enzymatic Conversion of Glutamine to Ammonia[1]

Ammonia is an end-product of both the enzymatic and non-enzymatic degradation of glutamine. In studying glutaminase activity it is not possible to distinguish between the ammonia formed by enzymatic and non-enzymatic reactions without performing substrate blanks. In previous studies[2,3] it became obvious that the optimum conditions required for rat renal glutaminase caused an appreciable non-enzymatic degradation of glutamine. Therefore a study was made of the rate of non-enzymatic ammonia production from glutamine with particular reference to the conditions which prevail when glutaminase activity is being assayed.

Ammonia was measured by the CONWAY microdiffusion technique[4]. The sample was alkalinized in the outer well of the unit by 1 ml of saturated potassium carbonate and the ammonia was trapped in the centre well by 1 ml of borate buffer. Except where otherwise stated, ammonia diffusion was allowed to take place at room temperature for 2 h.

The solid line in Figure 1 shows a typical recovery curve obtained with preformed ammonia. In this case the 10 μ equivalents of ammonia were recovered after 60 min. The broken line shows the rate of ammonia recovery when 120 μmoles of glutamine are added to the outer well in addition to the 10 μ equivalents of ammonium chloride. It will be noted that the curves separate after 5 min and that ammonia is being recovered from glutamine even after 3 h. These results show that when preformed ammonia is being analysed titration time is not critical after complete recovery but if glutamine is present in the sample titration time must be constant if the rate of degradation of glutamine is not known.

In glutaminase studies glutamine will usually be at 3 different temperatures for a significant period of time. The rate of glutamine degradation at these temperatures was then determined. For this type of experiment 120 μmoles of glutamine in a volume of 0.5 ml were put in sealed test-tubes with 1 ml of saturated potassium carbonate at various temperatures. At different time intervals a sample was removed from the incubator, quickly brought to room temperature and then poured into the outer well of the CONWAY unit. Ammonia absorption was allowed to take place for 2 h, which was more than sufficient time for diffusion of the largest amount of ammonia which was produced in these experiments. By bringing all samples to room temperature means that further degradation should be similar irrespective of the previous temperatures and that the rate of ammonia diffusion, which is temperature dependent[4], should be approximately equalized in all samples. The amount of ammonia recovered when 120 μmoles of freshly prepared glutamine were allowed to remain in the outer well for 2 h was subtracted from the values obtained. The corrected values are reported in Figure 2. It is obvious that at this high pH there is a considerable degradation of glutamine even at 0 °C. As the temperature was elevated there was an increase in the rate of degradation.

The effect of phosphate at various pH values and at 2 different temperatures is shown in Figures 3 and 4. At 20 °C (Figure 3) raising the pH from 5–10, in the absence of phosphate, increased the ammonia production by 50%. In the presence of 100 μmoles of phosphate a similar increase in the pH caused a 3-fold rise in ammonia production. This means that the effect of phosphate on the rate of glutamine degradation was greater at higher than at lower pH values. On the other hand, at 37 °C (Figure 4) phosphate had a similar effect on the rate of glutamine degradation at all pH values studied. However, at this higher temperature a rise in pH increased ammonia production more than it did at 20 °C. In the absence of phosphate an increase in the pH value from 5–10 caused a 50% rise in ammonia production at 20 °C and a 3- to 4-fold rise at 37 °C. Intermediate temperatures resulted in ammonia production between those values. Phosphate is normally employed in the assay of glutaminase 1. Due to its accelerating effect on non-enzymatic degradation of glutamine, even at 20 °C, at high pH values it will result in significant degradation of glutamine in the presence of potassium carbonate in the CONWAY unit.

All of the results reported here were obtained with 120 μmoles of glutamine. However, linear results were

[1] This work was supported by a grant from the Medical Research Council of Ireland.

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