

$2 \times 10^7$  cells and glucose (0.01 M) as substrate (0.5 ml/vessel). The bath temperature is 37 °C.

The Table shows the doses used, chosen on the base of preliminary tests carried out to establish the more active dose; it also shows the values of percentage inhibition of respiration of tumour and hepatic cells and the corresponding percentage inhibition of tumour development in vivo. The results are also shown as specific activity, in comparison with the DMB activity which is defined as equal to 1.

The biguanide derivatives which have been tested show a slight inhibitory action on the hepatic cells respiration. The inhibitory action is much stronger on the oxygen

consumption of the tumour cells; MFB, inactive in vivo, is practically so in vitro.

As a conclusion we can confirm that these biguanide derivatives, which have antitumoural activity in vivo, show an inhibitory action on respiration and that this action is selective toward tumour cells.

Therefore, we could propose the following hypothesis: the antitumoural action of such compounds could be caused by an inhibition of the synthesis of high energy phosphate compounds.

**Riassunto.** È stato studiato l'effetto di un gruppo di derivati biguanidici sulla respirazione in vitro di cellule tumorali ed epatiche. I composti esaminati sono tutti capaci di inibire, in vario grado, lo sviluppo del tumore ascite di Ehrlich del topo, tranne uno, privo di attività antitumorale in vivo. Tutti i composti mostrano di depri-  
mere lievemente il consumo di ossigeno delle cellule epatiche. I derivati attivi in vivo bloccano quasi completamente la respirazione delle cellule tumorali, mentre il composto inattivo ha un'attività assai inferiore. Si fa l'ipotesi che l'azione antitumorale dei derivati biguanidici si esplichi attraverso una inibizione dei processi respiratori collegati alla formazione di legami altoenergetici.

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Compound	mg/vessel	Percentage inhibition of O <sub>2</sub> uptake in vitro		Specific activity (tumour cells)	Percentage inhibition of tumour growth in vivo
		Hepatic cells	Tumour cells		
DMB	5	2	72	1.00	30
BB	2	23	91	3.16	49
PB	5	15	85	1.18	47
iPB	8	10	65	0.56	34
BuB	10	12	88	0.61	41
iBuB	7	22	78	0.77	51
MFB	10	7	24	0.17	—

## A New Antibacterial Agent Produced by *Streptomyces* sp. Ac<sub>6</sub>569

A number of streptothricin<sup>1</sup> type of antibiotic, e.g. geomycin<sup>2</sup>, racemomycin-O<sup>3</sup>, roseothricin<sup>4</sup>, streptolin<sup>5</sup> and viomycin<sup>6</sup> have been reported in the literature. A new antibacterial agent, belonging to this group of antibiotics, was isolated from the culture broth of *Streptomyces* sp. Ac<sub>6</sub>569, which showed high activity against bacterial and fungal test organisms.

The active material was produced in shake flasks in a medium containing soya peptone, 6 g/l; yeast extract, 2 g/l; KCl, 4 g/l; (NH<sub>4</sub>)<sub>2</sub> SO<sub>4</sub>, 5 g/l; KH<sub>2</sub>PO<sub>4</sub>, 0.4 g/l; CaCO<sub>3</sub>, 0.5 g/l; glucose, 2 g/l and pH was adjusted to 7.2 before sterilization. Elaboration of the antibiotic was measured by assay with *Staphylococcus aureus* and peak titres were usually obtained after 3–4 days incubation at 28 °C.

Isolation and purification of the active material was carried out by adsorption of the active material with Darco G-60 (25 g/l); elution of the active material with 0.1 N methanolic HCl, neutralization and concentration of the eluate under reduced pressure and purification by chromatography on a column of Darco G-60; Celite 545 (1:1) using 1% aqueous acetone (v/v) as the eluting agent.

The active material was found to be a tetra-acidic base and isolated in the form of hydrochloride as a pale yellow amorphous material, m.p. 216–218° (with decomposition). Probable molecular formula for the antibiotic was suggested as C<sub>23</sub>H<sub>43</sub>N<sub>9</sub>O<sub>8</sub> · 4HCl. No characteristic UV-absorption was observed in aqueous solution. IR-spectrum is indicative of the presence of carbonyl and guanidino type of grouping in the compound. The active material is

Comparative in vitro activity of Ac<sub>6</sub>569-sulphate with some of the known antibiotics

Test organism	Minimal inhibitory concentration µg/ml of			
	Ac <sub>6</sub> 569 sulphate	Kanamycin sulphate	Neomycin sulphate	Streptomycin sulphate
<i>Staphylococcus aureus</i> (sensitive)	3.0	3.0	0.125	3.0
<i>Staphylococcus aureus</i> (resistant)	5.0	3.0	0.25	3.0
<i>Staphylococcus albus</i>	0.5	1.0	0.25	5.0
<i>Bacillus anthracis</i>	8.0	0.5	0.25	2.5
<i>Bacillus subtilis</i>	3.0	1.0	1.0	1.0
<i>Bacillus megaterium</i>	2.5	1.0	1.0	3.0
<i>Proteus vulgaris</i>	12.0	3.0	6.0	10.0
<i>Escherichia coli</i>	0.5	0.5	0.25	0.5
<i>Aerobacter aerogenes</i>	3.0	3.0	1.0	3.0
<i>Pseudomonas aeruginosa</i>	40.0	50.0	20.0	30.0
<i>Salmonella typhosa</i>	6.0	4.0	2.0	15.0
<i>Candida albicans</i>	2.0	—	—	—

—, Not tested.

positive to ninhydrin, Sakaguchi, Elson-Morgan and negative to Fehling, Tollen and maltol.

The homogeneity of the active material was established by paper chromatography, paper electrophoresis and countercurrent distribution studies. The substance is readily soluble in water, partially in methanol and ethanol, and completely insoluble in acetone, ether, petroleum ether, *n*-butanol, benzene and chloroform. The antibiotic is stable at room temperature. A neutral solution of the antibiotic autoclaved at 15 pounds/square inch for 15 min lost about 60% of its original activity. A comparative assay of the active material with other known antibiotics are presented in the Table.

The toxicity test was carried out on mice using a dose of 25 mg/kg body weight i.v. There was no untoward symptoms or death within 72 h.<sup>7</sup>

**Zusammenfassung.** Ein neues, gegen Bakterien und Pilze wirksames Antibiotikum wurde aus dem *Streptomyces* sp. Ac<sub>6</sub>569 in der Form eines amorphen Hydrochlorids isoliert. Die Substanz ist wasserlöslich, aber fast unlöslich in organischen Lösungsmitteln, Schmp. 216–218° (Zer-

setzung); sie gehört zur Streptothricin-Gruppe der Antibiotika.

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## Photosynthesis and Respiration I. Effect of Light Quality on the Photorespiration in Attached Shoots of Spruce

Evidence of the occurrence of a photorespiration as a distinct process from dark respiration in plants, have been reported by a number of authors<sup>1–6</sup>. In the work presented below the effect of light quality on photorespiration was investigated. The white, red or blue lights were selected, in which illumination with used lights produced the similar rate of steady apparent CO<sub>2</sub> uptake. Under these conditions of illumination one would expect the action spectrum on CO<sub>2</sub> evolution in light if photorespiration is a light-sensitive process.

**Materials and methods.** Attached shoots of 4-year-old spruce seedlings *Picea glauca* Moench/Voss were used as experimental material. Seedlings were grown in pots in forest soil under natural conditions at the Petatawa Forest Experiment Station, Chalk River, Ontario, and brought to Queen's University. Attached shoots were sealed into a plexiglass photosynthesis chamber connected in a closed circuit system to an IR CO<sub>2</sub> analyzer according to the method described by LISTER et al.<sup>7</sup>. The volume of the system was 2.12 l and the rate of air flow was 1.8 l/min. The rates of apparent photosynthesis, photorespiration and dark respiration and concentration of CO<sub>2</sub> at CO<sub>2</sub>-compensation point were determined according to the methods described by TREGUNNA et al.<sup>2</sup>. The rate of apparent photosynthesis was determined at CO<sub>2</sub> concentrations from 360–250 ppm. The source of light was six 375 W Sylvania photoflood lamps (3200°K) filtered through 2 water screens. Between the light source and photosynthesis chamber, the red or blue cellulose acetate filters were introduced to modify the light quality. The transmission characteristics of these filters are given by TREGUNNA et al.<sup>8</sup>. Light intensity was measured by mean of radiometer YSI-65. The experiments were carried out at 25°C. The determinations of each process mentioned above was made in 2 consecutive light-dark cycles. The same plant material was first illuminated with white light

then with red, and blue, and vice versa. The data presented in this paper are typical for number of experiments.

**Results and discussion.** Data presented in the Table show that the rates of apparent photosynthesis under

The rates of apparent photosynthesis (APS), photorespiration (PR), dark respiration (DR) and CO<sub>2</sub> concentration at CO<sub>2</sub> compensation points (CO<sub>2</sub> comp.) in attached shoots of spruce illuminated with white, red or blue lights

Light quality	Light intensity in ergs/cm <sup>2</sup> per sec <sup>-1</sup>	μg CO <sub>2</sub> min/g fresh weight of needles			(CO <sub>2</sub> comp.) in ppm
		APS	PR	DR	
White	9 × 10 <sup>4</sup>	49.3	5.9	14.1	70
Red	1.2 × 10 <sup>5</sup>	46.8	4.7	14.6	68
Blue	5 × 10 <sup>5</sup>	46.8	18.2	13.8	170

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