sults, together with the concentration of test solutions, are summarized in the Table.

Effect of 1 and 10 mg/l of GA_3, 4, 7, 13, morphactin, and AMO-1618 alone and in combination is given. It was observed that sprouting starts almost simultaneously in control and treated 'eyes'. Morphactin inhibited sprout elongation, the inhibitory effect increasing with concentration. AMO, on the other hand, stimulated sprout growth at low concentration but was inhibitory at higher concentration. Gibberellins stimulated elongation of sprout growth, the stimulatory effect being more in higher concentration. The 4 gibberellins tested showed differential response in an order of $GA_7 > GA_3 > GA_4 > GA_{13}$. Water-treated 'eyes' showed minimum growth. The order of activity of different GAs observed here is different to that obtained by other workers in other biological tests[5-8].

Considering the interaction between different gibberellins and morphactin or AMO, the results showed that inhibition caused by morphactin is reversed only by GA_3 whereas AMO-induced inhibition was negated by all the gibberellins, although the length of sprouts when treated with different gibberellins in combination with AMO was less as compared to those treated with respective gibberellins alone.

The results indicate that morphactin in contrast to AMO – a gibberellin antagonist[9], is not the competitive inhibitor of action of all the gibberellins tested and is specific only to GA_3 in its mutual antagonist effect, because if it was not so that application of other gibberellins should have caused reversal of morphactin-induced inhibition as they did when used in combination with AMO-1618[10].

Length of sprouts, produced on excised dormant potato 'eyes' treated with different concentrations of gibberellins, morphactin and AMO-1618 alone and in combination, observed 14 days after the treatment

Treatment	Concentration (mg/l)	Mean length of sprout (cm ± S.E.)
Control	–	0.32 ± 0.07
	1.0	0.99 ± 0.36
GA_3	10.0	3.47 ± 0.94
	1.0	0.61 ± 0.20
GA_4	10.0	1.66 ± 0.48
	1.0	3.01 ± 0.90
GA_7	10.0	4.10 ± 0.80
	1.0	0.74 ± 0.30
GA_{13}	10.0	0.67 ± 0.40
	1.0	0.29 ± 0.07
Morphactin	10.0	0.26 ± 0.10
	1.0	0.69 ± 0.10
AMO-1618	10.0	0.30 ± 0.12
GA_3 + Morphactin	10.0 each	2.54 ± 0.82
GA_4 + Morphactin	10.0 each	0.34 ± 0.21
GA_7 + Morphactin	10.0 each	0.10 ± 0.02
GA_{13} + Morphactin	10.0 each	0.28 ± 0.11
GA_3 + AMO-1618	10.0 each	2.50 ± 0.69
GA_4 + AMO-1618	10.0 each	1.37 ± 0.43
GA_7 + AMO-1618	10.0 each	2.09 ± 0.68
GA_{13} + AMO-1618	10.0 each	1.01 ± 0.23

Zusammenfassung. Die Hemmwirkung, verursacht durch AMO-1618, auf das Auswachsen isolierter Kartoffelknospen lässt sich durch 4 getestete Gibberelline (GA_7, GA_3, GA_4 und GA_{13}) aufheben. Die Hemmung durch Morphactin wird jedoch nur von GA_3 aufgehoben. Morphactin scheint kein kompetitiver Inhibitor der getesteten Gibberelline zu sein.

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Bacterial Mutants of Hydrogenomonas Lacking Poly-β-Hydroxybutyric Acid

Poly-β-hydroxybutyric acid (PHBA) is a storage product of many aerobic and phototrophic bacteria. The accumulation of this lipid occurs preferentially when energy and carbon are available in excess and when growth is limited by the absence of utilizable compounds of nitrogen, sulphur or phosphorus. The lack of oxygen also results in the storage of PHBA. In *Hydrogenomonas* intracellular PHBA-deposition can amount to 65% of the cellular dry weight when the cells are incubated in a growth medium lacking a nitrogen source[1].

Mutants of *Hydrogenomonas H16* which are not able to store PHBA can be recognized and isolated by the following procedure: the cell suspension is distributed on nutrient agar plates (100–200 cells per plate) and incubated at 30 °C for 2 days. The colonies are transferred by replica plating to agar plates containing a medium low in nitrogen (0.005% ammonium chloride) and rich in carbon source (0.5% fructose). After 4 or 5 days incubation the colonies grown are treated with Sudanblack B: the agar plates are flooded with 10 ml of a 0.2% solution of Sudanblack B in 96% ethanol. After 20 min this solution is replaced by 10 ml of 96% ethanol, and after 1 min this is poured off. While the colonies of the PHBA-rich cells retain the dye and remain dark blue coloured, the colonies of the PHBA-poor or PHBA-free cells are completely decolorized during the differentiation process and appear

light grey or white. Cells of the PHBA-deficient colonies are isolated from the corresponding colonies on the master plate.

By applying this procedure, 4 mutants of *Hydrogenomonas H16* have been isolated which accumulate either no PHBA or less than the wild-type strain (Table). 1-nitroso-3-nitro-1-methyl-guanidine was used as the mutagenic agent. The amount of PHBA stored does not differ significantly with different carbon sources (fructose, gluconate, acetate, β-hydroxy-butyrate, carbon dioxide) indicating that the block of the biosynthetic pathway of PHBA in these mutants is located subsequent to the formation of β-hydroxybutyryl-coenzyme A.

One mutant (strain PHBA$^-$ 5) has been isolated employing the ^{32}P-suicide-technique[2,3]. Following nitrite treatment and further growth, the cells were allowed to accumulate PHBA. The cells were then washed and resuspended in a nutrient medium containing 1 mC ^{32}P-phosphate but no carbon source. During a growth period of 24 h, the cells containing PHBA incorporated ^{32}P-phosphate. The cells were centrifuged, resuspended in phosphate buffer and then stored frozen. After 2 weeks' time, the viable count had dropped by a factor of 10^{-5}. From this sample mutant PHBA$^-$ 5 has been isolated.

This mutant, however, exhibits pleiotropic effects. Although the mutants PHBA$^-$ 1 to 4 are identical with the wild-type strain with regard to growth rate, substrate utilization and other general properties, this mutant differs by exhibiting higher growth rates on solid media, a sensitivity to agitation of the liquid medium, excretion of gluconate when growing on glucose and in other properties. Experiments on respiratory control and the regulation of PHBA-synthesis are in progress.

PHBA-content of mutants and of the wild-type strain of *Hydrogenomonas* following incubation in the presence of fructose, gluconate, acetate or carbon dioxide + hydrogen in the absence of a nitrogen source

Strain or mutant, resp.	Amount of PHBA (% of dry weight) after incubation with			
	Fructose for 40 h	Gluconate for 23 h	Acetate for 23 h	$CO_2 + H_2$ for 26 h
H16 (wild-type)	65.3	27.7	37.2	35.8
H16 PHBA$^-$ 1	11.9	7.3	–	5.6
H16 PHBA$^-$ 2	–	8.6	8.2	1.5
H16 PHBA$^-$ 3	–	7.3	13.9	4.3
H16 PHBA$^-$ 4	–	0	0	0
H16 PHBA$^-$ 5	–	–	0	0

The cells were grown in a complete medium containing the substrates indicated. The suspension was centrifuged, and the cells were resuspended in a nitrogen-free medium containing the same substrates. After the incubation period the cells were harvested, washed, and freeze-dried. 100–400 mg of dry cell powder were used to gravimetrically determine the PHBA-content[4].

Zusammenfassung. Mutanten eines Bodenbakteriums (*Hydrogenomonas H16*), welche Poly-β-hydroxybuttersäure-Granula nicht zu synthetisieren und anzuhäufen vermögen, wurden isoliert. Anreicherungs- und Selektionsverfahren zur Isolierung solcher Mutanten werden beschrieben und die physiologischen Eigenschaften der isolierten Mutanten charakterisiert.

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Biological Observation in Quantitative Tests of *Nippostrongylus brasiliensis* Acting as Vector of *Trypanosoma brucei* or *Trypanosoma congolense*

In the last two decades, some investigators have demonstrated instances where helminths have been incriminated in transmission of disease agents, larvae of *Heterakis gallinae* transmit *Histomonas meleagradi's* (Graybill[1], Shope[2]), *Trichinella spiralis*, vector for virus of lymphocytic meningitis (Sylverton[3]), and the fluke *Nanophetus salmincola* for *Neorickettsia helminthoeca* (Phillips[4]).

Since then greater attention has been focused on the mechanism of immunity to protozoan and helminth parasites than on the primary effects on the host in a simultaneous infection. The present report therefore summarizes quantitatively the effect of concurrent infection of *Nippostrongylus brasiliensis* and *T. congolense* or *T. brucei* in laboratory rats in attempts to find out if this nematode might carry the protozoan to the host.

Materials and methods. The strain of *N. brasiliensis* and the trypanosomes species used in this study were maintained in the laboratory by weekly s.c. inoculations. A modification of Lincicome and Watkin's[5] method was used to prepare saline blood trypanosome suspension. Quantitative standardization of the trypanosome inocula was accomplished by use of haemacytometer and blood pipette.

The infective larvae of *N. brasiliensis* was obtained by a modified culture method of Yokogawa[6]. Each rat in-

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