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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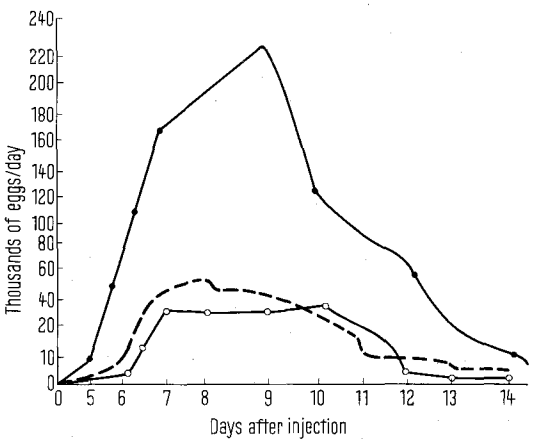
Data collected from inoculated rats (10 per group) with standard dose of 2000 *N. brasiliensis*, 2000 *T. congolense*, and 2000 *T. brucei*

Experiment No.	1	2	3	4	5
Rats infected with	*N. brasiliensis*	*N. brasiliensis* and *T. congolense*	*N. brasiliensis* and *T. brucei*	*T. congolense*	*T. brucei*
Total No. of eggs passed 5th–13th day after infection	920,000	195,000	155,000	–	–
Average adult worms recovered 9th day after infection	1318	869	754	–	–
Average spleen weight per rat	0.8–1.3 g	2.7–3.4 g	2.0–2.7 g	2.6–3.2 g	1.9–2.5 g
Liver size	Medium	Large	Medium–Large	Large	Medium
Average total white blood cells					
Days after infection: 5	15,500	9,900	10,200	10,800	11,000
6	15,000	11,100	11,400	10,500	12,500
7	14,600	11,800	13,500	12,300	14,760
8	13,800	12,750	14,700	14,550	15,120
9	14,000	13,650	15,200	16,000	17,000
Peak of trypanosomes population	–	2.3×10^8	2.5×10^8	2.6×20^8	2.8×10^8



Comparative average egg-count curves on rats concurrently infected with 2000 *N. brasiliensis* (●—●); 200 *N. brasiliensis* and 200 *T. congolense* (— —); 2000 *N. brasiliensis* and 2000 *T. brucei* (△—△).

fected received a total inoculum of 2000 *N. brasiliensis*. A total saline suspension of 2000 trypanosomes of each species were inoculated into each rat. Every inoculum was equally divided and injected s.c., i.p. i.v. and given orally.

Five sets of experiments (10 rats per set) were conducted. The first set were injected concurrently with 2000 *T. brucie* and 2000 *N. brasiliensis*. The second group were initially inoculated 2000 *T. congolense* and then subsequently 2000 *N. brasiliensis* 4 days later. The third set were infected with 2000 *T. brucei* only, while the fourth group were challenged with 2000 *T. congolense* only. A fifth set were injected 2000 *N. brasiliensis* alone. The degree of parasitemia was seen daily by examining fresh blood drops (from rat's tail) and Giemsa stained preparation under microscope. Total white blood cell counts were also made employing certified pipettes and haemacytometer. Average spleen and liver weight ratio were also determined. The course of *N. brasiliensis* in both single and double infections were followed by daily egg counts and adult worm population at autopsy.

Results and discussion. Five sets of experiments were conducted to test the biological effect of concurrent infection of *T. brucei*, *T. congolense* on *N. brasiliensis* and to ascertain further whether the nematode would transmit the trypanosomes during its cyclic phase. The significant results are summarized in the Table.

In experiments 2 and 4, *T. congolense* attained its peak propagation on the 8th day while that of *T. brucei* in experiments 3 and 5 was on the 10th day after infection. Selected rats from each group were sacrificed for worm count, and with a dissecting microscope thorough examinations of the nematode worms were made for trypanosomes. Trypanosomes were found only in 5 of the recovered adult worms from experiment 3, and in 1 from experiment 2. The total eggs passed (5th–13th day) in the concurrent infections were considerably less than in the single infections. The number of adult worms recovered in experiments 2 and 3 were much less than in experiment 1.

Increase in splenic weights were shown by the concurrently infected rats than by those singly infected. The degree of parasitemia in the single infections with the trypanosomes was almost similarly pronounced in the double infections with the trypanosomes and *N. brasiliensis*. These differences were attributed to a physiological factor. However, the alterations in the total white blood cells in experiments 2–5 seem to be in conformity with *T. congolense* and *T. bruccei* characteristics. 4 of the rats autopsied revealed stunted worms in the lungs (experiments 2–3). 35% of the rats infected in experiments 2–5 after the peak of infection looked emaciated with remittent fever and loss of appetite. The reduced egg production and/or loss of worms could have been influenced by the presence of trypanosomes, or probably the beginning of immunity in the host to *N. brasiliensis*.

Résumé. Des rats ont été infectés par des Trypanosomes soit directement soit par l'intermédiaire de *Nipponostrongylus*, Nématode parasite. Le degré de la parasitémie produite dans le premier cas fut à peu près aussi élevé que dans le second. Des différences apparurent dans le poids de la rate et l'altération des leucocytes.

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