

Table 1. Evolutionary lineage of mice in the Siwaliks.

Formation	Murid genera	Age
Pinjor Formation	<i>Mus</i> sp.	Early Pleistocene of Pakistan (Jacobs <sup>5</sup> )
Tatrot Formation	<i>Mus</i> sp.	Late Pliocene of India (Patnaik in this work)
Dhok Pathan Formation	<i>Mus auctor</i>	Late Miocene of Pakistan (Jacobs <sup>5</sup> )
Nagri or Dhok Pathan Formation	<i>Progonomys debruijini</i>	Late Miocene of Pakistan (Jacobs <sup>5</sup> )
Chinji Formation	<i>Antemus chinjiensis</i>	Middle Micoene of Pakistan (Jacobs <sup>5</sup> )

molar) with an asymmetrical 'X' pattern at the anterior portion of the tooth and the absence of labial cingulum. In  $M^1$  (first upper molar), anterostyle is antero-posteriorly compressed and is posterior relative to the anterocone and the posterior cingulum is reduced.

The present *Mus* sp. can be differentiated from *Mus auctor* and *Progonomys debruijini* of Late Miocene of Pakistan on the basis of the absence of labial cingulum in  $M_1$ . It differs from *Karnimata* and *Parapodemus* of Late Miocene of Pakistan by its smaller size, absence of anterior mure, labial cingulum and medial anteroconid in  $M_1$ . It can be differentiated from *Parapelomys* of Late Miocene of Pakistan by the presence of pattern 'X' and the absence of anteromedial cingulum in  $M_1$ . It differs from *Golunda* of Pleistocene of Pakistan by the absence of medial anteroconid and labial cingulum in  $M_1$ . The present molars compared with those of *Mus booduga* (recent field mice found in India<sup>7</sup>) showed that both are similar to each other, except that in the  $M_1$  of the recent ones, the hypoconid and the entoconid are more strongly connected and the 'X' pattern is more asymmetrical.  $M^1$  of *Mus booduga* has a less elongate anterior portion and a small prestyle.

*Antemus chinjiensis* of Middle Miocene of Pakistan is considered to be the oldest murid known so far and its low crowned nature of  $M^1$ , weakly connected cusps and weak labial cingulum in  $M_1$  marks it to be more primitive than *Progonomys debruijini* and *Mus auctor* of Late Miocene of Pakistan<sup>5</sup>. *P. debruijini* is considered as ancestral to *Mus auctor* in having cusps in the chevron less strongly connected and anterostyle more posteriorly placed<sup>5</sup>. *P. debruijini* can be considered as more primitive than the present *Mus* sp. in having a  $M^1$  with posterior cingulum (from primitive characters of murids<sup>5</sup>). *Mus auctor* is more primitive than the *Mus* sp. described here with an anterostyle more posteriorly placed, anterior portion less wide in  $M^1$ , a prominent labial cingulum, a hypoconid and an entoconid less strongly connected. The present specimens are very similar to those of *Mus* sp. reported from Early Pleistocene of Pakistan<sup>5</sup>, but these specimens can be considered as ancestral to the *Mus* sp. from Pakistan, as they occur in older sediments of Siwaliks. *Mus* sp. described here can be linked (Table 1) with the above mentioned genera, as all of them share the character of an anterostyle rather posterior in position than those in other murid genera.

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## A method for the estimation of food consumption by insect parasitoids

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Considering the growth of parasitized host after parasitization, a new method was developed to estimate the bioenergetic parameters of parasitic insects. *Spodoptera exigua* Hubner parasitized by *Apanteles prodeniae* Viereck was taken as the model system. *A. prodeniae* consumes 16.5 J, excretes 0.53 J, assimilates 15.97 J and produces 11.37 J. Rates of feeding, assimilation, production and metabolism can be estimated using this method.

ESTIMATION of food available to the parasitoids, which infect actively growing stages of their hosts, is difficult for the following reasons: (i) After parasitization, the host ingests less food and grows slowly, (ii) on any particular day after parasitization, the weight or the energy content of the parasitized host represents not only the weight or the energy content of the host but also of the parasitoid developing inside. However, for parasitoids infecting non-growing stages of their hosts such as egg or pupa, estimation of host energy available

to the parasitoid is relatively easier. Prakash and Pandian<sup>1</sup> determined the host energy available for *Sarcophaga banksi* Senior-White parasitizing the eggs of the spider *Argiope pulchella* Thorell by considering the number of eggs present in the egg sac during parasitization and the number of spiderlings eclosed from the sac; they used the linear relationship between the egg sac area and the number of eggs present in the sacs to estimate the number of eggs initially present in the sac; considering the number of eggs ingested and the energy content of a single egg, the host energy ingested by *S. banksi* was estimated. Using the relation between the wet weight and the energy content of the host, Howell and Fisher<sup>2</sup> calculated the host energy available to the parasitoid, *Nemeritis canescens* Grover during parasitization. However, they failed to consider the growth realized by the host after parasitization.

In the present method, the host energy available to the parasitoid is derived from the growth curves of the parasitized host and of the parasitoid. Energy content of the host when it attains maximum growth can be noted from the easily estimatable growth curve of the parasitized host. Unfortunately, estimation of growth of the parasitoid is difficult. Estimation of the energy content of the freshly hatched parasitoid larva is difficult owing to its very small size rendering its location in the host-haemolymph difficult. As location, isolation and manoeuvring of the parasitoid larva is possible after 4 or 5 days of parasitization, the larvae may be dissected out from the host, counted and weighed once in 2 days after the fourth day of parasitization and the mean weight of a single larva at 2 day intervals determined. The parasitoid larvae do not egest the faeces but retain it in their hindgut and egest as meconium during pupation<sup>3</sup>. Hence, the energy content of the terminal larva also includes the energy equivalent of the meconium. Assuming the energy content of the freshly hatched larva to be negligible, growth ( $P$ ) may be calculated by subtracting the energy content of the meconium ( $Me$ ) from the energy content of the terminal larva ( $TL$ ):

$$P = TL - Me.$$

Considering the energy content of the parasitoid larva on different days after the fourth day of parasitization, a growth curve for the parasitoid may be drawn.

Subtracting the energy content of the parasitoid from that of the parasitized host when it attained its maximum growth, the host energy available to the parasitoid can be estimated (Figure 1). Energy ingested by the parasitoid larva may be calculated by subtracting the energy content of the host remains (carcass) from the host energy available. After realizing maximum growth, the host ingested less food and egested most part of it as faeces<sup>4</sup>. Energy content of the host decreases due to rapid utilization of the host tissue by

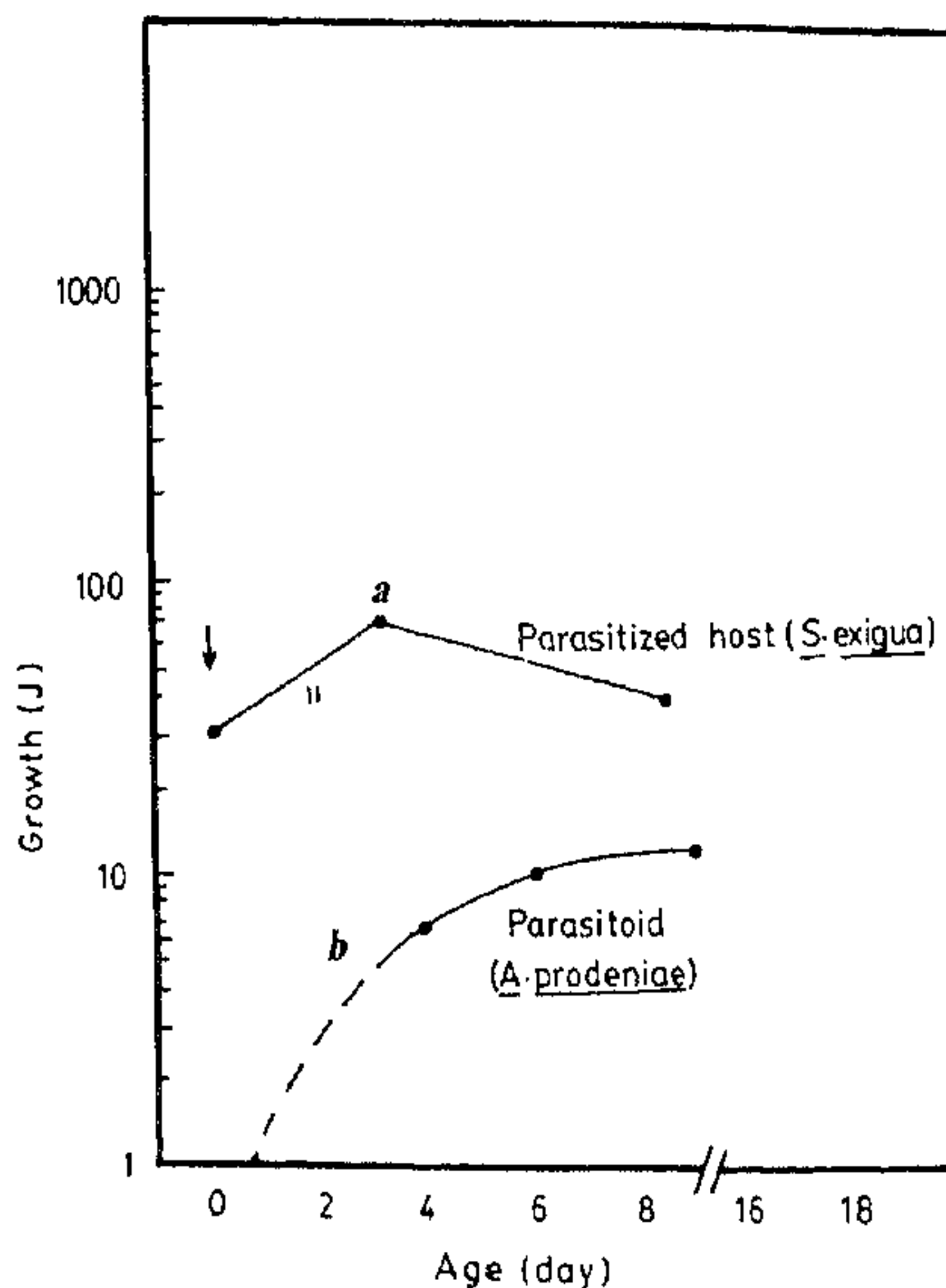


Figure 1. Estimation of food energy available to the parasitoid. Roman numeral indicates host larval instar and arrow indicates stage of parasitization. *a*, Energy content of the host at its maximum growth; *b*, Energy content of the parasitoid larva at the time of maximum growth of the host.

Note: Energy density of parasitized *S. exigua* larva (25 larvae/estimation; 3 estimations) and that of the parasitoid larva (*A. prodeniae*; 80 larvae/estimation; 1 estimation) was estimated using a Parr 1421 (Moline, USA) semimicro bomb calorimeter. Multiplying the dry weight of the insects with their corresponding energy density, biomass was converted into energy values.

the parasitoid. Therefore, the difference between the energy contents of the host and of the parasitoid, when the host attained its maximum growth, may be treated as the host energy available to the parasitoid. This procedure of determining host energy available to the

Table 1. Food consumption and utilization by *Apanteles prodeniae* parasitic on *Spodoptera exigua*.

Host energy available (J)	$64.7 \pm 5.2 - 5.2 \pm 0.3 = 59.5 \pm 3.2$	
Carcass (J)	$42.8 \pm 2.6$	
Consumption (J)		$16.5 \pm 1.2$
Meconium (J)	$0.53 \pm 0.1$	
Assimilation (J)		$15.97 \pm 1.0$
Production	$11.37 \pm 0.6$	
Metabolism (J)		$4.60 \pm 0.2$
Feeding rate (J/g/day)	$1.746 \pm 0.098$	
Assimilation rate (J/g/day)	$2.690 \pm 0.104$	
Production rate (J/g/day)	$1.203 \pm 0.077$	
Metabolic rate (J/g/day)	$0.487 \pm 0.030$	
Assimilation efficiency (%)	$96.8 \pm 5.8$	
Gross production efficiency (%)	$68.9 \pm 3.7$	
Net production efficiency (%)	$71.2 \pm 4.0$	



parasitoid takes into account the increase in the energy content of the host subsequent to parasitization. It also accounts for the energy expended on metabolism by the host. Table 1 provides estimates of the host energy available and that ingested by *A. prodeniae* parasitizing on *S. exigua*.

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## Nutritional value of the newly isolated *Saccharomyces cerevisiae* of palm wine

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An analysis of the newly isolated *Saccharomyces cerevisiae* showed that this strain could be a better feed/food supplement in terms of nutrition. The treatment of this strain with the mutagen *N*-methyl-*N'*-nitro-*N*-nitroso guanidine increased the thiamine content 3-fold, thus rendering the organism more nutritive.

YEASTS are used as food/feed supplement because of its protein, amino acid and vitamin B contents. Among the yeasts, *Candida utilis*, *Kluyveromyces fragilis* and *Saccharomyces cerevisiae* are generally used as nutritional sources<sup>1</sup>. When yeasts were given as feed supplement to animals, there was an improvement in weight, egg size and increased disease resistance<sup>2-4</sup>.

The palm sap or wine obtained from palm trees used as a beverage particularly in South India, harbors many yeast strains. These have not been properly exploited for nutritional purposes. We have isolated several yeast strains from palm wine of local palm trees. One of the isolates was identified as *S. cerevisiae* which gave better biomass and ethanol yield compared to others (Table 1). This strain was further evaluated for its nutritive value in terms of cell protein, amino acid composition and thiamine (vitamin B<sub>1</sub>). The effect of *N*-methyl-*N'*-nitro-*N*-nitroso guanidine (MNNG), a potent mutagen, on the nutritional improvement of this strain was also studied.

*S. cerevisiae* isolated from palm wine on YEPD medium (yeast extract-1%, peptone-2% and dextrose-2%) was stored on the slants layered with paraffin oil at 4°C.

The biomass of the culture was estimated by drying the cells to a constant weight. Protein was estimated by determining the nitrogen content of the cells using the Microkjeldahl method<sup>5</sup>. The thiamine and riboflavin contents were estimated by chemical methods<sup>6,7</sup>. The amino acid composition of the cellular protein was determined by subjecting it to HCl hydrolysis and analysing the amino acid contents using an automatic Beckman amino acid analyser (119 CL).

The cells were treated with 0.2 mM MNNG (Sigma) for 210 min by the method described by Fahrig<sup>8</sup>. The cells after treatment were washed free of the mutagen and plated on YEPD medium with 1.5% agar. About 20 colonies were selected at random after incubation at 28°C and analysed for their nutritive value.

The newly isolated yeast strain of *S. cerevisiae* was found to contain 47% protein and 180 µg and 40 µg of thiamine and riboflavin/g dry weight of cells respectively. These values are comparable to the U.S. National Formulary (N.F × 1) 1960<sup>1</sup>.

In general, the contents of some of the essential amino acids of the total cellular proteins were

Table 1. Ethanol and biomass yield of various isolates of yeasts after 72 h incubation at 28°C in a shaker.

Source of isolation	Predominant yeast	Ethanol yield in g/g glucose	Biomass yield in g/g sugar/l
Grape (Anabshahi)	<i>Hansenula</i> spp.	0.22 ± 0.005	0.3 ± 0.08
Grapes (Anabshahi)	<i>Hansenula</i> spp.	0.15 ± 0.005	0.2 ± 0.06
Seedless grapes	<i>Hansenula</i> spp.	0.23 ± 0.01	0.3 ± 0.01
Seedless grapes	<i>Hansenula</i> spp.	0.19 ± 0.005	0.19 ± 0.01
Grapes (Bangalore blues)	<i>Hansenula</i> spp.	0.175 ± 0.005	0.19 ± 0.09
Soil	<i>Saccharomyces</i> spp.	0.24 ± 0.005	0.31 ± 0.009
Soil	<i>Saccharomyces</i> spp.	0.25 ± 0.007	0.32 ± 0.008
Soil	<i>Saccharomyces</i> spp.	0.23 ± 0.006	0.28 ± 0.009
Palm wine (Toddy)	<i>S. cerevisiae</i>	0.27 ± 0.005	0.34 ± 0.009
Palm wine	<i>S. cerevisiae</i>	0.25 ± 0.004	0.30 ± 0.01
Palm wine	<i>S. cerevisiae</i>	0.24 ± 0.005	0.3 ± 0.009
Palm wine	<i>S. cerevisiae</i>	0.18 ± 0.005	0.19 ± 0.01
Palm wine	<i>S. cerevisiae</i>	0.16 ± 0.004	0.17 ± 0.009
Palm wine	<i>S. cerevisiae</i>	0.3 ± 0.005	0.4 ± 0.01
Mutagen-treated sample 17	<i>S. cerevisiae</i>	0.325 ± 0.006	0.42 ± 0.01

Mean of 5 individual experiments carried out in duplicates.