Effect of dietary antagonists and corresponding nutrients on growth and reproduction of the flour mite (*Acarus siro* L.)

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"Durch die Zugabe von Antiwuchsstoffen können wir nachweisen, daß das Wachstum der betreffenden Organismen gehemmt wird, und daß diese Hemmung durch die Zugabe des entsprechenden Wuchsstoffes wieder aufgehoben wird."

Niels Nielsen, 1945*

Abstract. The flour mite *Acarus siro* L. (Acaridae, Astigmata) was reared on an axenic diet with the addition of various nutrient antagonists, with and without supplementation by the corresponding nutrients. The deficiency symptoms induced by dietary antagonists, and the reversibility of the former by nutrient administration, indicated that folic acid, riboflavin, thiamine, niacin, pyridoxine, biotin and a sterol are essential for the growth and reproduction of the flour mite. It was also demonstrated that the population density and generation sequence of this species can be suppressed to the level of acaristasis by nutrient antagonists, owing to inhibited nutrient utilization.
Key words. Flour mite; *Acarus siro*; requirements for folic acid, riboflavin, thiamine, niacin, pyridoxine, biotin, sterol; dietary antagonists; acaristasis.

Distribution and economic importance

The minute, sac-like, slow-moving flour mite *Acarus siro* (Linnaeus 1758), also known as *Tyroglyphus farinae* (Linnaeus 1758), is probably a native species of the palearctic region, where it has become a common pest of stored foodstuffs including cereal flours, damaged grain, yeast, moulds, dried fruit and cheese (fig. a). It feeds preferentially on cereal flours, penetrating the latter to a depth of 5–10 cm. It causes severe losses by soiling due to accumulation of dead mites, cast cuticles and malodorous faeces, and discoloration, making those foodstuffs inedible as well as unsuitable for baking[1,2]. Synanthropic birds and rodents are likely to promote the dispersal of flour mites among storage premises[3].

Griffiths[1,22] separated the *Acarus siro* complex into three distinct species, viz. *A. farris, A. immobilis* and *A. siro*, which can be readily recognized in the adult and hypopal stage. *A. siro* is usually inhabiting storage premises where it mainly feeds on processed cereals, while *A. farris* and *A. immobilis* are incapable of consuming processed cereals and thus prefer field habitats[1].

Respiration and water conservation

The acarid flour mite belongs to the order Astigmata. Species of this order usually have a thin and scarcely sclerotized cuticle, devoid of tracheae and stigmata[4]. Gas exchange and water balance in *A. siro* thus depend on the relative permeability of the exocuticle, which is adjacent to the epidermis, and on the humidity of the atmosphere. The flour mite responds to a regimen of 85–90% relative humidity and 20–25 °C by an optimal rate of reproduction[3], and eliminates most of its nitrogenous waste in the form of insoluble guanine[5].

Growth and reproduction

Growth of the juvenile stages of *A. siro* commences with a hexapod larva (lacking secondary sexual characters) which moults to an octopod protonymph (provided with a rudimentary genital opening). The latter develops to a tritonymph (provided with an incomplete genital aperture) which eventually moults to a mature female or male, ready for copulation or spermatophore transfer approximately one day after ecdysis. Adverse environmental conditions lead to the formation of a hypopus, a rarely-occurring, non-feeding deutonymph which is inserted between the protonymphal and tritonymphal stages. Hypopi lack an oral cavity, possess a rudimentary gnathosoma and reduced legs (3rd and 4th pair), and are provided with 8 anal and 2 genital suckers[1,2,4,6]. When environmental conditions are favourable, the development (from egg to imago) requires 15–17 days, including a brief period of quiescence (~ 1 day) following each juvenile stage[7]. The literature reveals considerable fluctuations in fecundity, mainly due to different temperatures: the number of eggs laid per female and life-time (on a diet of wheat germ) varies from 93–463 at ~ 14 °C, 76–408 at ~ 21 °C, 80–256 at ~ 28 °C[8] to an average of 119 at ~ 25 °C[9].

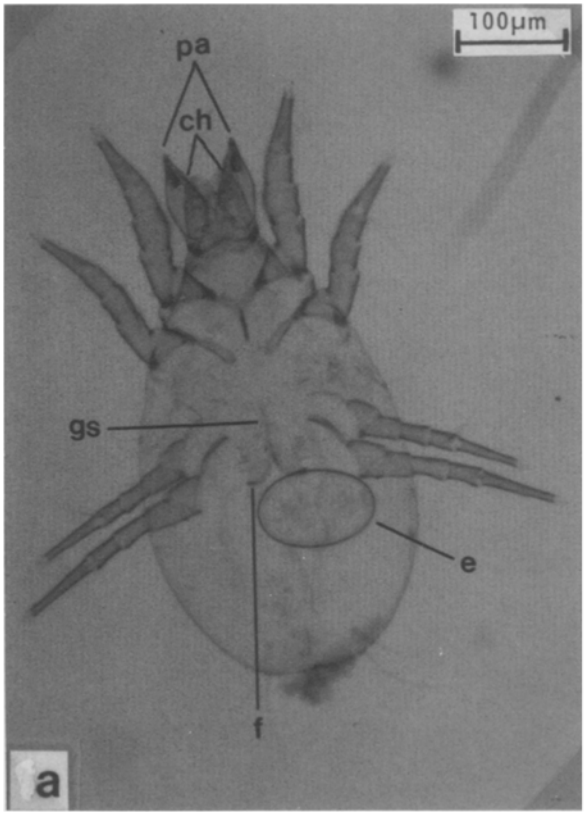
Nutrient antagonists and arthropod species

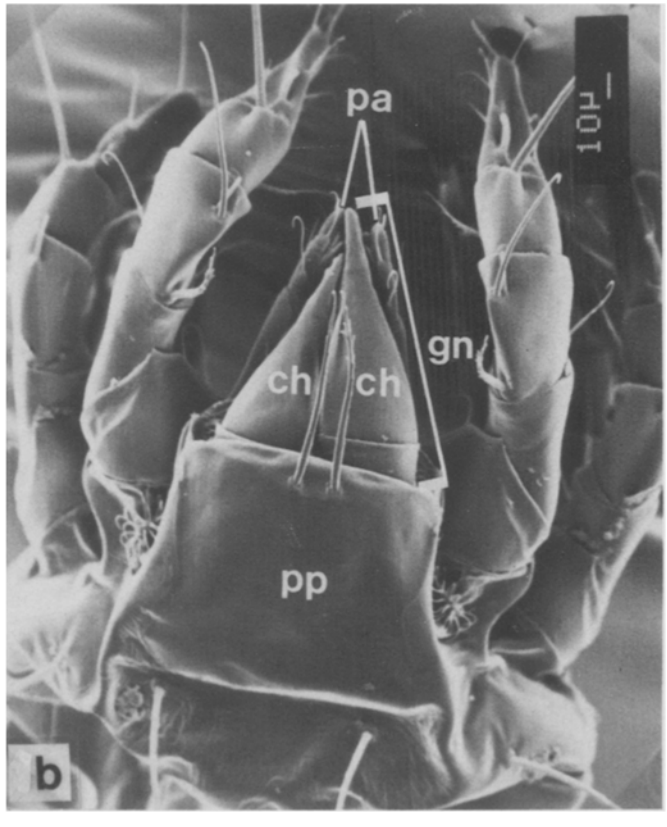
It has been shown that certain substances that are structural ana(homo)logues or precipitants of indispensable dietary components, antagonize the utilization of essential nutrients in several insect species[10] and two mite species[11]. For example, addition of sodium bicarbonate to biscuits results in a substantially depleted thiamine level and makes them inadequate as larval food for the flour beetle *Tribolium confusum*[12]. Dietary administration of 5×10^{-3}% of 4-aminofolic acid (an analogue of folic acid) or 35 unit % of avidin (a biotin precipitant) to the hide beetle *Dermestes maculatus* causes symptoms of vitamin deficiency and high larval mortality[13]. Wool fabrics impregnated with ~ 0.5%

of imidazol (a niacin antagonist) induces suppressed feeding and digestion as well as larval mortality of the carpet beetle *Anthrenus flavipes* (= *A. scrophulariae*)[14]. Impregnation of bean leaves *(Phaseolus lunatus)* with 10^{-1}% of pyrithiamine (a competitive inhibitor of thiamine) leads to reduced growth of larvae of the Mexican bean beetle *(Epilachna varivestis)* and death due to starvation[15]. Development of housefly larvae *(Musca vicina)* is inhibited in the dietary presence of 10^{-1}% cholesteryl chloride and 5×10^{-3}% cholesterol (1 : 0.05), whereas the availability of 10^{-1}% cholesteryl chloride and 3×10^{-2}% cholesterol (1 : 0.3) permits growth and pupation of this species[16]. It follows that elimination or modification of certain dietary components of foodstuffs, causing nutritional deficiencies in harmful arthropods, could be a useful approach to prophylactic pest control[11, 13, 17].

Scope of work

Insect species feeding mainly on cereal flours (e.g. *Tribolium confusum, Ptinus tectus)* depend on the dietary presence of biotin, choline, folic acid, m-inositol, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine and a sterol; omission of any one of those nutrients from the diet is known to result in hampered growth, and mortality[18, 19]. Since the food substrates of the flour mite closely resemble those of the above insect species, it was expected that growth and/or reproduction of *A. siro* could be curtailed by dietary uptake of antagonists of the essential nutrients mentioned above. We therefore investigated the effects of nine dietary antagonists and corresponding nutrients on growth and reproduction of the flour mite. Production of deficiency symptoms following ingestion of a dietary antagonist, as well as reversal of those effects upon feeding the corresponding nutrient, indicated a dietary requirement for the latter.





a Magnified, ventral view of a gravid female of *Acarus siro* L., ~ 7 days old (embedded in Heinze's medium). The mobile gnathosoma bears conspicuous palps (pa) and chelicerae (ch); the outlines of the latter are evident below the large, pointed palps. A completely developed egg (e) is found in the oviduct near the genital aperture, which is situated between the coxae of the third and fourth pair of legs. The outline of an embryonal larva can be recognized through the egg shell. The elongated genital slit (gs), enclosed by to diverging folds (f) delivers the eggs. Approximately 14–20 spermatophores are usually transferred to a female's receptaculum seminis, within a period of 10 days. Length of the idiosoma is ~420 µm, while the egg dimensions are ~ 120 × 70 µm.

b Scanning electron micrograph showing the dorsal ganthosoma (gn) and propodosomal plate (pp) of a female flour mite, ~ 7 days old. During feeding, flour mites hold their gnathosoma at an obtuse angle to the propodosomal plate. Each cone-shaped chelicera (ch) ends in two serrated digits, one of which is fixed, while the other is movable. In the course of ingestion, both digits operate like shears gouging and scraping particles of food which are then transferred to the oesophagus by means of a suctorial pharynx. The palps (pa), being provided with sensory hairs, are used for the location of food; they can be partly seen below the chelicerae.

Materials and methods

Stock diet and rearing procedure

Experimental flour mites were reared on ground yeast, singly and in groups, under axenic conditions in the absence of the microbial species which usually grow in the habitat of *A. siro*[5].

In the course of feeding, the gnathosoma of flour mites is turned downwards, whereby the tips of the chelicerae are brought in contact with the food. Each chelicera comprises a broad basal shaft tapering off to a fixed digit, which articulates with a movable digit; both are provided with serrated edges (fig. b). The two pairs of toothed digits work in a vertical plane like shears, gouging and scraping particles of food which are passed on to the oesophagus by means of a suctorial pharynx. The above feeding equipment is not suitable for the penetration of the glossy and hard coat of dried seeds, whereas it can be readily used in order to obtain food particles from cracked cereal grains or cereal flours. Since the flour mite ingests its food in the form of solid particles, dried and finely ground baker's yeast (particle diameter 6–18 µm) was used as the stock diet for *A. siro*.

The flour mites were continually bred in a precision incubator (BK 5060 E, Heraeus, 6450 Hanau 1, FRG) at a constant temperature of 22 ± 0.2 °C, relative humidity of 85–90% and complete darkness. The stock diet was finely ground yeast *(Saccharomyces cerevisiae)* obtained from Gist-Brocades, Delft, Holland. The ground yeast was shown to have an approximate content of 520 mg protein, 260 mg carbohydrates, 120 mg fat, 20 mg sterols (mainly ergosterol) and 55 mg water per gram of diet. It also contained the following B-vitamins (µg/g of diet): p-aminobenzoic acid 40, biotin 0.15, choline 5000, folic acid 22, inositol 2200, niacin 500, pantothenic acid 200, pyridoxine 30, riboflavin 50 and thiamine 50. Microbial species known to thrive in the habitat of *A. siro* may be a source of nutrients for the latter[5]; growth of those microorganisms was therefore prevented by the addition of 10 mg butyl p-hydroxybenzoate and 50 µg chloramphenicol per gram of diet.

Groups of 3 female and 3 male flour mites were confined to glass-covered, gypsum-lined concave chambers (volume ~ 500 µl) with ~ 50 mg of stock diet; they were maintained under axenic conditions for 30 days following eclosion. Constancy of the relative humidity in the chambers was checked daily by means of indicator strips (cobalt chloride) attached to the cover lid, while observation of the mites was facilitated by means of impregnation of the gypsum layer (1.5 g) by Sudan Black B (1 mg) and methyl p-hydroxybenzoate (1.5 mg).

Experimental diets

The highly purified nutrients and nutrient antagonists employed (tables 1 and 2) were obtained from Sigma Chemicals Co. (St. Louis, USA). Stock diets were supplemented by individual nutrient antagonists with and without addition of the corresponding nutrients. The molar ratios between nutrient and corresponding antagonist were usually 1 : 0 (control diet), 1 : 1, 1 : 10 and 1 : 100. Yeast sterols and digitonin were tested at molar ratios of 1 : 0, 1 : 0.1, 1 : 0.3, 1 : 1 and 1 : 10.

Homogeneous distribution of the compounds added to the test diets was accomplished by grinding and mixing 2-g batches of the former in a ball mill (Pulverisette 7, Fritsch Ltd., 6580 Idar-Oberstein, FRG) using 15 ml containers (Achat) provided with 4 Achat spheres (diameter 12 mm). The particle diameter of experimental diets was 6–18 µm.

Food uptake by juvenile and imaginal stages was determined by feeding 5-mg batches of experimental or control diets to 100 neonate larvae until adult emergence, or to 100 adults of either sex for a period of 10 days, and subsequently weighing the remains of the diets, and the adult flour mites.

Growth and mortality of juvenile stages

Fifty mated females (3–5 days old) of the parental generation were allowed to oviposit during 6 hours. The eggs were distributed among 5-mg batches of experimental and control diets in gypsum-lined chambers at a rate of one egg per chamber. Each experimental and control diet was tested with 4 replicates of 40 eggs. Experiments on the influence of nutrient antagonists on the growth of *A. siro* were always performed with parallel control tests using untreated stock diet.

Living and dead larvae, protonymphs, tritonymphs and newly emerged adults were observed through the transparent covers of the chambers and recorded at 12-h intervals, using a binocular microscope (magnification: 10–25 ×). The illumination involved in such recordings did not have any deleterious effects on the flour mites, which are known to lack compound eyes as well as ommatidia[20]. The median duration of growth (from egg to imago) and the percentage of mortality of juvenile *A. siro* due to ingestion of insalubrious diets were calculated (table 1).

Suppression of population density

Twelve females and twelve males, after emergence from resting tritonymphs of the parental generation, were maintained as single pairs in gypsum-lined chambers, each containing ~ 50 mg of an experimental or control diet. The proportions of adult females, males, eggs and juvenile stages of both filial generations on each diet were determined after a period of 30 days. Twelve pairs were tested on each of the diets, modified as shown in table 2.

Oviposition and larval hatching

Fourteen pairs of both sexes (~ 1 day old) of the parental generation were maintained in 14 gypsum-lined chambers each containing 5 mg of an experimental or control diet. Recording of oviposition and larval hatch-

ing were carried out by transferring the adults at one-day intervals to new gypsum-lined chambers containing the same diet as before. The number of eggs and hatching larvae of the filial generation were recorded for a period of 30 days following adult emergence, and the mean rates of fecundity and fertility then calculated (table 2).

Evaluation of results

Records of duration of growth from egg to imago (40 individuals/replicate, table 1) on experimental and control diets failed to show a normal distribution, despite the relatively homogeneous age of the eggs used (0.1–6.0 h after oviposition). Therefore medians and first (25%) and third (75%) quartiles were evaluated, in order to reflect the asymmetrical distribution as adequately as possible. Mann and Whitney's U-test (based on Wilcoxon's test) was employed for the determination of insignificant ($\alpha = 0.05$) and significant differences ($\alpha = 0.01$) in growth on experimental and control diets[21].

The size of populations arising from single pairs on an experimental diet (within 30 days) was compared with that of the populations obtained on a control diet (12 pairs/replicate), also using Mann and Whitney's U-test. The tabulated results (table 2) refer to a population decrease as % of control, being either significant ($\alpha = 0.01$) or not ($\alpha = 0.05$).

The parameter 'reproduction' in table 2 reveals the number of larvae per female on experimental diets as a percentage of the respective control data (14 repetitions/diet) using the above statistical procedure.

The main lesions following ingestion of certain dietary antagonists were a mortality of more than 60% of the juvenile stages, the suppression of oviposition by more than 60%, and the prevention of larval hatching in more than 60% of the eggs laid. The procedure of binomial probability[21] was employed for testing the significance ($p < 0.01$) of juvenile mortality and suppressed fertility on experimental versus control diets.

Table 1. Influence of nutrient antagonists on duration of growth (egg to imago) of *Acarus siro* L. Results are based on 160 eggs (filial generation) per nutrient antagonist, nutrient and molar ratio, in 4 replicates of 40 eggs. m = complete mortality of juvenile stages, s = significant ($\alpha = 0.01$), ins = insignificant ($\alpha = 0.05$) as compared to control (Mann and Whitney's test). Further details are given under 'Materials and methods'.

Nutrient	Nutrient antagonist	Molar ratio (1)	Duration, days Median	Quartile, days First	Third	Growth retardation
Yeast sterols	Digitonin	1 : 0.1	15	14	17.5	ins
		1 : 0.3	21	19	29	s
		1 : 1	m	-	-	-
		1 : 10	m	-	-	-
Folic acid	4-Aminofolic acid	1 : 1	22	19	25	s
		1 : 10	26	19	28	s
		1 : 100	m	-	-	-
Riboflavin	Quinacrine	1 : 1	15	13	17	ins
		1 : 10	18	15	21	s
		1 : 100	19	16	21	s
Thiamine	Pyrithiamine	1 : 1	18	15	20	s
		1 : 10	19	18	22	s
		1 : 100	m	-	-	-
Niacin	3-Acetylpyridine	1 : 1	19	15	21	s
		1 : 10	20	15	29.5	s
		1 : 100	m	-	-	-
Pyridoxine	4-Deoxypyridoxine	1 : 1	14	13	19	ins
		1 : 10	14	13	15	ins
		1 : 100	15	12	21	ins
Pyridoxine, niacinamide	Isonicotinic acid hydrazide	1 : 1	15	13	25	ins
		1 : 10	17	13	21	ins
		1 : 100	18	15	22	s
Biotin	Avidin	1 : 1	15	12	19	ins
		1 : 10	18	16	21	s
		1 : 100	m	-	-	-
Pantothenic acid	D-Pantoyltaurine	1 : 1	16	16	18	ins
		1 : 10	17	15	20	ins
		1 : 100	18	16	20	s
None	None	Control	15	13	17.5	-

(1) nutrient : nutrient antagonist.

Table 2. Suppression of population density and reproduction of *A. siro* due to nutrient antagonists, and reversal of their action by corresponding nutrients. The data on population density are based on the offspring of 12 single pairs, recorded on a given diet after 30 days, while the data on reproduction are shown by the number of neonate larvae/female descending from 14 single pairs on a given diet throughout life. Population density is the quotient of the mean density on an experimental diet and the mean density on the control diet, expressed as % of control density. Reproduction is the quotient of the mean number of neonate larvae/female on an experimental diet and the respective data on the control diet, expressed as % of control reproduction. s = significant ($\alpha = 0.01$) as compared to control (Mann and Whitney's test). Further details are given under 'Materials and methods'.

Nutrient	Nutrient antagonist	Molar ratio (1)	Population density (2)		Reproduction (2)		Main lesions	Mode of action
Yeast sterols	Digitonin	1 : 0.1	100		100		-	
		1 : 0.3	29	s	86		-	
		1 : 1	2	s	25	s	jm, so	r
		1 : 10	0	s	0	s	-	
Folic acid	4-Aminofolic acid	1 : 1	20	s	76		-	
		1 : 10	10	s	60	s	jm, so	r
		1 : 100	0	s	0	s	-	
Riboflavin	Quinacrine	1 : 1	24	s	58		-	
		1 : 10	17	s	41	s	so	r
		1 : 100	10	s	20	s	jm	
Thiamine	Pyrithiamine	1 : 1	45	s	90		-	
		1 : 10	5	s	58	s	jm, sfer	r
		1 : 100	0	s	0	s	-	
Niacin	3-Acetylpyridine	1 : 1	47	s	75		-	
		1 : 10	38	s	42	s	jm, so	r
		1 : 100	0	s	0	s	-	
Pyridoxine	4-Deoxypyridoxine	1 : 1	60		90		-	
		1 : 10	33	s	38	s	so	r
		1 : 100	7	s	20	s	jm	
Pyridoxine, niacinamide	Isonicotinic acid hydrazide	1 : 1	74		73		-	
		1 : 10	18	s	32	s	sfer	irr
		1 : 100	5	s	10	s	jm	
Biotin	Avidin	1 : 1	70		90		-	
		1 : 10	58	s	57	s	jm, sfer	r
		1 : 100	0	s	0	s	-	
Pantothenic acid	D-Pantoyltaurine	1 : 1	95		100		-	
		1 : 10	85		85		-	r
		1 : 100	80		70		-	
None	None	Control	100		100		-	-

(1) nutrient : nutrient antagonist, (2) percent of control, - = none, jm = mortality of juvenile stages, so = suppressed oviposition, sfer = suppressed fertility, r = reversal and irr = irreversal of nutrient antagonist effects by corresponding nutrient.

Results

Bionomical data

The adult flour mites (fig. a) attained a length of 360–440 µm (males) and 420–580 µm (females) when reared on the stock diet at 22 ± 0.2 °C, 85–90% r.h. and in complete darkness. The average data given below were obtained under these environmental conditions.

The white eggs were ellipsoidal and smooth, had a relatively high yolk content and attained a length of 100–120 µm (fig. a). An incubation period of 4.5 days was required in order to induce larval hatching. The larvae grew up within 3.4 days to a length of 180–220 µm. The protonymphs reached a length of 240–300 µm within the following 2.2 days and the tritonymphs developed during 1.8 days, attaining a length of 340–420 µm. Moulting occurred during three quiescent periods (1.1 days/period) between subsequent juvenile stages as well as between the tritonymphal and imaginal stages. The median duration of growth from egg to imago was 15 days. The average life span of adult females was 35 days. When 20 female and 20 male *A. siro* were maintained as single pairs throughout their lives, an average number of 98 ± 24 eggs was laid per female.

Food uptake

Comparison of the quantitative food uptake by juvenile and imaginal stages did not reveal a significant difference between the control diet and the diets containing nutrient antagonists.

Retardation of growth by nutrient antagonists

Table 1 reveals that the median duration of growth of *A. siro* is 15 days on the control diet. When certain nutrient antagonists were added to the diet, growth required significantly longer. Dietary uptake of 4-aminofolic acid at a ratio of 10 : 1 or 1 : 1 to folic acid prolonged the growth period by 11 or 7 days, respectively. Dietary uptake of digitonin and yeast sterols (mainly ergosterol) at a ratio of 0.3 : 1 resulted in a growth prolongation of 6 days. Ingestion of 3-acetylpyridine at 10 : 1 of niacin prolonged the growth period by 5 days, and the consumption of 3-acetylpyridine at 1 : 1, pyrithiamine at 10 : 1 or

quinacrine at a ratio of 100 : 1 of the corresponding nutrients all caused a growth prolongation of 4 days. Growth periods were extended by 3 days as a result of dietary administration of either pyrithiamine at 1 : 1, quinacrine at 10 : 1, avidin at 10 : 1, isonicotinic acid hydrazide at 100 : 1 or D-pantoyltaurine at 100 : 1 of the corresponding nutrient. A growth prolongation of less than 3 days as compared to the median growth period on the control diet was considered to be insignificant.

Suppression of population increase and reproduction due to nutrient antagonists

The population arising from a single pair of *A. siro* comprised 421 ± 56 individuals (including eggs, juvenile and adult stages) after 30 days on the control diet. Since a generation lasted from 13 days (first quartile) to 17.5 days (third quartile) (table 1), the above population resulted from at least two subsequent and partly overlapping generations.

The population density of *A. siro* was significantly reduced when certain dietary antagonists were consumed by the flour mites. The existence of four groups, reflecting different levels of reduced population densities, can be inferred from table 2. *Group a):* complete suppression of progeny resulted from dietary administration of 10 : 1 of digitonin and yeast sterols as well as 100 : 1 of either 4-aminofolic acid and folic acid or pyrithiamine and thiamine or 3-acetylpyridine and niacin or avidin and biotin. *Group b):* marked decrease in the population density (up to 20% of the control population) caused by dietary uptake of either 1 : 1 of digitonin and yeast sterols or 4-aminofolic acid and folic acid, by 10 : 1 of quinacrine and riboflavin, or pyrithiamine and thiamine, or isonicotinic acid hydrazide and niacinamide, as well as by 100 : 1 of 4-deoxypyridoxine and pyridoxine. *Group c):* moderately decreased population densities (21–40% of the control population) resulted from ingestion of 0.3 : 1 of digitonin and yeast sterols, 1 : 1 of quinacrine and riboflavin, or 10 : 1 of either 3-acetylpyridine and niacin or 4-deoxypyridoxine and pyridoxine. *Group d):* somewhat reduced population densities (41–60% of the control population) caused by dietary uptake of 1 : 1 of either pyrithiamine and thiamine or 3-acetylpyridine and niacin or 4-deoxypyridoxine and pyridoxine as well as by 10 : 1 of avidin and biotin.

The reproductive potential of female flour mites receiving a control diet throughout life was 93 ± 22 neonate larvae/female. Reproduction could be considerably inhibited or even abolished as a result of dietary uptake of certain nutrient antagonists (table 2). Reproduction of *A. siro* was completely inhibited by ingestion of 10 : 1 of digitonin and yeast sterols as well as by 100 : 1 of either 4-aminofolic acid and folic acid or pyrithiamine and thiamine or 3-acetylpyridine and niacin or avidin and biotin. Marked suppression of reproduction (up to 20% of the control level) resulted from dietary uptake of 100 : 1 of either quinacrine and riboflavin or 4-deoxypyridoxine and pyridoxine or isonicotinic acid hydrazide and niacinamide. Moderate suppression (21–40% of the control level) was caused by ingestion of 1 : 1 of digitonin and yeast sterols as well as 10 : 1 of either 4-deoxypyridoxine and pyridoxine or isonicotinic acid hydrazide and niacinamide. Some reduction (41–60% of the control level) resulted from dietary uptake of 10 : 1 of either 4-aminofolic acid and folic acid or quinacrine and riboflavin or pyrithiamine and thiamine or 3-acetylpyridine and niacin or avidin and biotin.

Causes of suppressed population density

The observed reductions in population density were due both to growth retardation and to certain lesions. Mortality of juvenile stages was found to result from several nutrient antagonists fed to *A. siro* in excess of the corresponding nutrients (tables 1 and 2). Complete larval mortality was induced by dietary uptake of 10 : 1 of digitonin and yeast sterols as well as 100 : 1 of either 4-aminofolic acid and folic acid or avidin and biotin. Complete tritonymphal mortality resulted from ingestion of 100 : 1 of either pyrithiamine and thiamine or 3-acetylpyridine and niacin, while a tritonymphal mortality of 70–95% was induced by dietary uptake of 100 : 1 of either quinacrine and riboflavin or 4-deoxypyridoxine and pyridoxine or isonicotinic acid hydrazide and niacinamide.

Considerably reduced oviposition (5–30% of the control level) was noticed in female flour mites which received diets containing 1 : 1 of digitonin and yeast sterols as well as 10 : 1 of either 4-aminofolic acid and folic acid or quinacrine and riboflavin or 3-acetylpyridine and niacin or isonicotinic acid hydrazide and niacinamide. Fertility was suppressed to a similar extent (5–30% of the control level), when pyrithiamine and thiamine or isonicotinic acid hydrazide and niacinamide or avidin and biotin were continuously fed to female *A. siro* at a molar ratio of 10 : 1.

Male flour mites which had consumed dietary antagonists, retained a reproductive potential similar to that of males on the control diet, viz. 14–20 spermatophores/male within 10 days.

Suppressed fecundity and fertility as well as mortality of the juvenile stages, combined with retarded growth, are the major causes of reduction in population increase due to dietary antagonists. The predominance of either growth retardation or the above lesions seems to depend mainly on the quantitative relationship between the active antagonists and nutrients ingested. The fact that the tested nutrient antagonists failed to affect spermatophore formation in male flour mites demonstrates that the action of the former is restricted to the tissues of growing stages and reproducing females.

Reversibility of inhibition

The above symptoms of nutrient deficiency induced by sustained ingestion of individual dietary antagonists in

A. siro (tables 1 and 2) could be readily reversed upon feeding ergosterol, folic acid, riboflavin, thiamine, niacin, pyridoxine or biotin as multiples of 10 and 100 of the corresponding nutrient antagonists. The suppressing influence of D-pantoyltaurine was relatively weak, but could be reversed by pantothenic acid in excess. Neither pyridoxine nor niacinamide was capable of counteracting the effect of isonicotinic acid hydrazide.

Discussion

Nutritional value of ground yeast under axenic conditions compared with wheat germ

The nutritional value of ground yeast *(Saccharomyces cerevisiae)* for flour mites resembles that of wheat germ, for which *A. siro* has a dietary predilection. The median growth period (from egg to imago) at 22 °C and 85–90% r.h. was found to be ~ 15 days on the former (table 1) and ~ 13 days on the latter diet[23]. Ground yeast and wheat germ diets provide high levels of protein. *A. siro* has a digestive fluid with high proteolytic activity[24].

The fecundity of flour mites reared on milled yeast at 22 °C and 85–90% r.h., (98 ± 24 eggs per female and lifetime) was similar to the fecundity rate (119 eggs per female and lifetime) observed on wheat germ at 25 °C and 89% r.h.[9]. Maximal oviposition by flour mites also depends on the frequency of mating (one spermatophore being transferred at each mating). When flour mites were maintained as single pairs on ground yeast (22 °C, 85–90% r.h.), males transferred 14–20 spermatophores to females within 10 days. Griffiths and Boczek[25] reported that the first mating of *A. siro* leads to an average deposition of 78 eggs per female, while the second mating results in an additional output of 30 eggs per female.

Action of nutrient antagonists

It has been shown that sustained ingestion of either digitonin, 4-aminofolic acid, quinacrine, pyrithiamine, 3-acetylpyridine, 4-deoxypyridoxine, isonicotinic acid hydrazide or avidin by *A. siro* results in severe deficiency symptoms ranging from growth retardation and mortality of juvenile stages to suppressed fecundity and fertility. Mortality of larvae and tritonymphs is likely to be the consequence of cumulative inaccessibility of an essential nutrient. The above deficiency symptoms could be prevented by adding the corresponding nutrients to the diet, except for niacinamide or pyridoxine which were incapable of counteracting the influence of isonicotinic acid hydrazide.

The reversibility of the deficiency symptoms due to ingestion of digitonin, 4-aminofolic acid, quinacrine, pyrithiamine, 3-acetylpyridine, 4-deoxypyridoxine or avidin permits the conclusion that ergosterol (or other yeast sterols), folic acid, riboflavin, thiamine, niacin, pyridoxine and biotin are indispensable nutrients for the flour mite, being required both for growth and reproduction. Interestingly, the development of the copra mite, *Tyrophagus putrescentiae*, was also restrained by 4-aminofolic acid, quinacrine, pyrithiamine, α-picolinic acid and partly by cholesteryl chloride[11], but as information on the reversibility of the effects by corresponding vitamins and sterols is not available, the nutrients required by *T. putrescentiae* and *A. siro* cannot be compared.

The mode of action of the nutrient antagonists tested can be partly explained in biochemical terms[26–28].

4-Aminofolic acid, a close structural analogue of folic acid (p-[(2-amino-4-hydroxy-6-pteridylmethyl)-amino]-benzoylglutamic acid) differs from the latter by having an amino group on carbon 4 instead of the hydroxyl group. It acts by inhibiting dihydrofolic acid reductase, the enzyme which converts 7,8-dihydrofolic acid into 5,6,7,8-tetrahydrofolic acid. The relationship between antagonist and vitamin is competitive in *Streptococcus faecalis* and noncompetitive in *Escherichia coli.*

Quinacrine (an antimalarial, also called Atebrin or Mepacrine) is 3-chloro-7-methoxy-9-(1-methyl-4-diethyl aminobutylamino) acridine. Its configuration somewhat resembles that of riboflavin (7,8-dimethyl-10-ribitylisoalloxazine), and it blocks cytochrome reductase and D-amino acid oxidase, i.e. enzymes which have flavin adenine dinucleotide as their prosthetic group. This inhibition can be reversed by riboflavin-5′-phosphate.

Pyrithiamine is a structural analogue of thiamine (3-[4-amino-2-methylpyrimidyl-5-methyl]-4-methyl-5-[β-hydroxyethyl]thiazolium hydrochloride), from which it merely differs in possessing a pyridine ring instead of the thiazol ring. Pyrithiamine is a competitive inhibitor of the enzyme synthesizing carboxylase.

The structure of 3-acetylpyridine is like that of niacin (pyridine-3-carboxylic acid), except for the substitution of an acetyl group for the carboxyl group. 3-Acetylpyridine was found to react with DPN in an exchange reaction, catalyzed by diphosphopyridine nucleotidase, in order to form the acetylpyridine analogue of DPN. The latter may function as a cellular inhibitor of natural DPN.

The configuration of 4-deoxypyridoxine is almost identical with that of pyridoxine (2-methyl-3-hydroxy-4,5-bis[hydroxymethyl]pyridine); the only difference is the presence of a methyl group instead of the hydroxymethyl group on C4. 4-Deoxypyridoxine, known as a growth inhibitor of several pyridoxine-dependent species of microorganisms, is phosphorylated in the latter to an ester which competes with pyridoxal phosphate for the apoenzyme of certain amino acid decarboxylases.

Isonicotinic acid hydrazide is a structural derivative of niacinamide (pyridine-3-carboxylic acid amide) and has a hydrazide group on C4 instead of the C3-amide group; its structure also resembles that of pyridoxine. Hence it may antagonize the action of both niacinamide and pyridoxine. It inhibits the growth of *Mycobacterium tuberculosis*, probably as a result of its incorporation into an unnatural analogue of DPN.

D-Pantoyltaurine has the same configuration as pantothenic acid (D(+)-N-[-2,4-dihydroxy-3,3-dimethylbutyryl]-β-alanine) except for the carboxylic group, which is replaced by a sulfonic acid group. D-Pantoyltaurine interferes with the incorporation of pantothenic acid into coenzyme A in certain microbial species, but fails to induce symptoms of pantothenic acid deficiency in hamsters, mice and rats.

Unlike the structural antagonists of vitamins, digitonin and avidin act as nutrient precipitants. Precipitants can be used to exclude certain nutrients from a diet[10]. It has been known for more than eighty years that sterols with a free 3-hydroxyl group in the β-configuration form an equimolar, scarcely soluble complex with the steroid saponin digitonin, unless they possess a 10-methyl group in the epi-position[29]. The sterols of *Saccharomyces cerevisiae* mainly comprise ergosterol (Δ5,7,22-ergostatrien-3β-ol) and minor amounts of zymosterol (Δ8,24-cholestadien-3β-ol), 5-dihydroergosterol (Δ7,22-ergostadien-3β-ol), 24-dehydroergosterol (Δ5,7,22,24-ergostatetraen-3β-ol), ascosterol (Δ8,23-ergostadien-3β-ol) and fecosterol (Δ8,24,(28)-ergostadien-3β-ol)[30].

The basic glycoprotein avidin is known to form a solid complex with biotin (cis-hexahydro-2-oxo-1H-thieno-[3,4]imidazole-4-valeric acid) at a molar ratio of 4:1[31,32]. Since this complex is resistant to the action of proteolytic enzymes, avidin-bound biotin cannot be resorbed by the intestinal tract of flour mites.

In order to ascertain whether the tested compounds (tables 1 and 2) do indeed exert antivitamin or antisterol effects on flour mites, experiments on the reversibility of the effects were carried out. They revealed a pronounced improvement of growth and reproduction in response to elevated amounts of folic acid, riboflavin, thiamine, niacin, pyridoxine, biotin or ergosterol in the presence of a constant level of the corresponding antagonist. It follows that the nutrients mentioned above are essential for growth and reproduction of the flour mite. A requirement for pantothenic acid could not be shown, because D-pantoyltaurine failed to cause marked deficiency symptoms. It remains to be clarified which vitamins are required by *A. siro* in addition to folic acid, riboflavin, thiamine, niacin, pyridoxine, biotin and ergosterol. Interestingly enough, *Tenebrio molitor* and *Ephestia kuehniella*, which preferentially consume cereal flours, require the same B-vitamins[19] and sterols[33,34] as the flour mite.

Acaristasis

One of the aims of this study was to find out whether the growth of flour mite populations can be curtailed sufficiently to avoid economic damage. It appears desirable to have a specific term to designate a situation where the density of acarid infestations on foodstuffs is kept sufficiently low. Analogously to 'insectistasis'[35], 'acaristasis' (Acari = mites, stasis = stagnation) denotes a (flexible) state in which a population of a harmful mite species is restricted to the degree that food storage, or plant growth, are not significantly impaired.

The results of the above investigation demonstrate the possibility of inducing acaristasis in flour mite populations by antagonists of nutrients which are indispensable to the mites. Malnutrition caused by a nutrient antagonist may, according to the amount ingested, prolong the growth period, suppress fecundity and fertility, or cause untimely mortality in preimaginal stages of *A. siro*. These effects eventually lead to markedly reduced population densities and to a decreased number of generations per time unit. It is evident that it will only be possible to use dietary antagonists to produce acaristasis if there is a sufficiently wide gap between the dosage required to cause nutritional deficiency in flour mites and the amounts permissible in foodstuffs intended for human or animal consumption.

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Presence of a partial urea cycle in the leech, *Poecilobdella granulosa*

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Abstract. Ornithine carbamoyltransferase (OCT) and arginase, but not arginine synthetase (AS), were detected in the body wall and gut tissues of the leech. The activities of these enzymes were not altered by starvation. The high activity of arginase in body wall is probably due to the association of the latter with botryoidal tissue. Hirudineans, which evolved from oligochaete ancestors, appear to have lost the citrulline-arginine segment of the urea cycle due to their ammonotelic mode of nitrogen excretion.
Key words. Urea cycle; leech; botryoidal tissue; hirudineans; evolution.

Annelids are primarily ammonotelic [4, 5]. However, earthworms are known to switch over to ureotelism during starvation and considerable quantities of urea are present in the excreta of leeches and coelomic fluid of polychaetes [5]. Since uricolytic enzymes are absent in all members of Annelida [4], purine degradation does not seem to contribute to the excretory urea in these animals. The observation of Robin et al. [6] that in leeches labelled amidine from arginine appears in urea prompted Needham [5] to postulate the possible presence of urea cycle enzymes in hirudineans. However, the earthworm, *Lumbricus terrestris*, is the only annelid where a functional urea cycle has been demonstrated to date [7, 8]. Representatives of Polychaeta and Hirudinea have not been hitherto investigated for urea cycle enzymes. We report here that only a partial urea cycle is present in the Indian cattle leech, *Poecilobdella granulosa*, and that the activities of these enzymes, unlike those of the earthworm, are not altered in response to starvation stress.

A single blood meal sustains the cattle leech, *P. granulosa*, for as long as six months before the effects of starvation are noticeable [9]. Leeches were collected after a blood meal and used in experiments within a couple of weeks (fed group) or eight months later (starved group). Animals with empty and blood-filled alimentary canals were discarded from the fed and starved groups respectively. Body wall and gut tissues were homogenized at 0 °C in 9 volumes of 0.1 % acetyltrimethylammonium bromide. Acid-washed sand (BDH, England) was used in the homogenization of body wall. The homogenates were centrifuged at 4 °C, 15,000 × g for arginase and 4000 × g for ornithine carbamoyltransferase (OCT) and arginine synthetase (AS; combined activity of argininosuccinate synthetase and argininosuccinate lyase). The enzyme activities in the supernatants were assayed, after appropriate dilution, as described earlier [10, 11].

Arginase, which catalyzes the conversion of arginine to ornithine and urea, and OCT, which catalyzes the conversion of ornithine and carbomylphosphate to citrulline, were present in both body wall and gut tissue of the leech (table). The AS system, which forms arginine from citrulline, aspartate and ATP, could not be detected in either tissue. More concentrated homogenates and longer incubation periods were employed to confirm the apparent absence of this enzyme system in leech tissues. Our inability to detect AS in leech tissues is not due to the inadequacy of the assay method employed, because the method yielded enzyme activity levels in frog and rat livers similar to those reported in the literature [12].

OCT and arginase activities in leech tissues were lower than those in the earthworm gut [7]. In the leech, the activity levels of arginase were 11-fold higher in the body wall than the gut, while the levels of OCT were similar in both tissues (table). The two tissues in the polychaete, *Arenicola marina*, exhibit about the same level of arginase activity [13]. In the earthworm, on the other hand, both enzyme activities are several times higher in the gut than in the body wall [7, 14]. Most of the arginase activity in the earthworms is concentrated in the chloragog tissue, which forms a compact layer surrounding the gut, while OCT is