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SPECIES AND STAGE INTERACTION IN THE FEEDING BEHAVIOUR OF VECTORS OF CHAGAS DISEASE (THE IMPORTANCE OF DETERMINANTS IN PLANNING FOR GREATER EFFICACY AND STANDARDIZATION OF XENODIAGNOSTIC PROCEDURES)

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SUMMARY

Information on the interaction of the factor of species and developmental stage in the feeding behaviour of vectors of Chagas Disease is given. Experimental facts are liberally seasoned with speculations on their possible usefulness in the elaboration of a xenodiagnostic procedure, which may be as sensitive in the chronic phase of the disease as it has been found to be in the acute phase. The demonstration that young immature forms are capable of considerable blood uptake and that female bugs take up more blood than male bugs indicates that attention should be given to these determinants in considering a model insect for xenodiagnosis. The fact that vectors of Chagas Disease, fed once only, can live for long periods of time, indicates that "booster" meals allowed on succeeding occasions after feeding on chagasic patients are not necessary. However, it raises the question, whether the life of *T. cruzi* approaches the life expectancy of the invertebrate host under starving conditions and, whether the sequence of irreversible morphological changes that the flagellate undergoes in the midgut of the bug is independent of external food supply. The demonstration that blood uptake is an amount appropriate to developmental stage of the insects enables them to perform the function of molting upon a single meal suggests that at least some of the insects fed on chagasic patients will undergo transformation to the next stage. This raises the immediate question as to whether infectivity in the invertebrate host is limited to the developmental stage in which it is acquired, or if parasites consumed by one stage can survive through transformation of the host to the next stage.

INTRODUCTION

Investigators are in agreement that xenodiagnosis is outstandingly efficient in demonstrating parasitemia in the acute phase of Chagas Disease, even when trypanosomes are not easily detected in direct blood smears.

Although an intensive program has followed [6, 11, 18, 19] to refine this test for greater efficacy in the chronic phase of the chronic phase of the disease, with a low degree of parasitemia, it continues to give at most 56% positive results in chagasic patients, as demonstrated by LIMA et al. [10].

It should be emphasized that fundamental in the evaluation of any diagnostic procedure is the process under trial and the material on which the process is tried. In performing

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xenodiagnosis much concern has been given to the use of the last developmental stages of the vector. Thus, for many years attention was focused on 4th and 5th instar nymphs as the most efficient transport host for the flagellates. Although, possibly due to the considerable volumes of blood sucked by these stages, it may also be a reflection of insufficient knowledge on which to base vulnerability of different developmental stages. Because the tests worked with the material used in the acute phase of the disease, there was little impetus to look for a more sensitive material that might serve to detect flagellates in the chronic phase of infection.

While it could be shown that insect specificity and ample blood supply, constituting the meal of the insect, were of importance, it seems unlikely that only this accounts for the efficiency of the test. Other factors, such as interaction of age and sex of the insect host in the susceptibility to infection must be considered in the choice of a suitable host to standardize and elevate the sensitivity of the test.

Since no information is available, it would be of use to know whether different developmental stages of the invertebrate host differ in their susceptibility to *T. cruzi* infection. Younger stages may be of value much the same as is young age in the susceptibility of vertebrate host to infection [3, 9].

Also lacking are data on the influence, if any, of sex on the "life cycle" of the flagellate in the invertebrate host. A possible example of infectivity correlated with sex is offered by BURTT [2] who in experiments on the transmission of *T. rhodesiense* to the tsetse fly found about twice as many male as female flies developing salivary gland infection. It should, however, be emphasized that the greater proportion of early deaths occurring among infected females may have been responsible for these results.

An understanding of selective bugs for xenodiagnosis in the chronic phase of the disease, or in the evaluation of the effect of chemotherapy, cannot advance far until factors mentioned above have been adequately studied.

This reasoning prompted us to investigate the feeding behaviour of various bug species, leaving for the next step studies on the importance of this determinant in the invertebrate-parasite relationship.

This paper, in particular, shows the species and developmental stage interaction in the feeding behaviour of vectors of Chagas Disease. Attention is given to the initial of the feeding behavioural response in newly-born insects. It also evaluates the molting capability after a single blood meal. In addition it gives information on the survival capability of insects under starvation conditions.

MATERIAL AND METHODS

Insects utilized in these experiments were derived from field material which had passed one full generation in the laboratory.

The origin of vector species their maintenance, the rearing techniques have already been described by the Author [14, 15]. Briefly *Triatoma infestans, Triatoma sordida* and *Rhodnius neglectus* originated from the State of São Paulo. *Triatoma brasiliensis* and *Panstrongylus megistus* were collected in the State of Ceara and *Triatoma maculata* was brought in from the State of Pernambuco, Brazil.

Insects used in these experiments were kept in wide-mouth beakers, 600 ml capacity. Discs of white filter paper were used in the bottom of the jars and rectangles of filter paper folded accordian style were placed vertically, thus enabling the insects to crawl up to the top when exposed to chickens for feeding. The tops of the jars were covered with cheese-cloth or nylon stockings fastened with elastic bands. For feeding, jars containing bugs were placed in contact with chickens which had their feathers removed at the point of feeding, as shown in Fig. 1. The tests were conducted at room temp. and relative humidity of 65 to 70%.

EXPERIMENTS

Feeding behaviour of newly hatched insects — The capability of newly born insects to cope with external food supply can easily be evaluated by the difference in body-shape of fed and unfed individuals.

Fig. 1 — Feeding of bugs

Groups of insects, born within 24 hours, are introduced into beakers and exposed to chickens for one hour. The engorded insects are then transferred to clean beakers and their number recorded. The unfed are retained in the original beaker, exposed on successive days thereafter, until the cumulative number of fed ones reaches over 95%.

The results given in Table I show that practically all individuals within any species demonstrated certain indifference to food on the first day after hatching. On the third day, however, the number of fed insects, within the three best feeding of the six species, varied from 83 to 100%.

Species of insects seems to represent the principal factor that affected the capability of early feeding. *T. sordida* was the first to reveal an interest in external food supply, as shown by the 96% fed on the 2nd day after hatching. The other species in descending order were: *T. infestans* with 92% fed on the 3rd day, *R. neglectus* and *P. megistus* with 97-98% engorged on the 4th day and *T. maculata* and *T. brasiliensis* with 95-96% fed as late as on the 7th day after hatching.

The effect of feeding on generation time will be taken up in the discussion.

Blood uptake — The objective of the following experiments was to obtain information on the interaction of developmental stage and species on the blood uptake, the criterion of which was weight difference before and after feeding for each of the successive developmental stages within various species. Adult males and females were tested separately, since other tests by the Author [11] and by HACK [7] have shown differences in feeding behaviour.

TABLE I

Feeding capability of new-born nymphs shown by the time-course of the accumulated percentage of engorged specimens

Species	Sample size	Cumulative percentage of engorged first instar nymphs on days after emergence							
		1st	2nd	3rd	4th	5th	6th	7th	8th
T. infestans	232	0	29.7	91.8	100	—	—	—	—
T. sordida	292	4.4	95.8	100	—	—	—	—	—
T. brasiliensis	144	0	1.3	13.1	89.4	63.8	89.5	95.1	100
T. maculata	161	0	31.6	52.1	73.9	78.8	82.6	95.6	100
P. megistus	95	3.1	29.4	52.6	96.8	100	—	—	—
R. neglectus	97	0	7.2	83.5	97.9	97.9	97.9	97.9	97.9

TABLE II

Ninety five percent confidence limits for means of vector body weight and vector blood uptake

Developmental stage (instar)	Sample size	Body weight (mg)*		Blood uptake (mg)**	
		L_1 and L_2 of the mean	$\overline{Y}$	L_1 and L_2 of the mean	$\overline{Y}$
		Triatoma infestans			
1st	47	1.638 — 1.656	1.65	5.37 — 5.90	5.64
2nd	14	5.4 — 7.2	6.3	18.0 — 24.5	21.2
3rd	12	15 0 — 17.3	16.2	55 1 — 100.2	77.6
4th	10	42.7 — 50.7	46.7	77.8 — 163.6	120.7
5th	8	78.2 — 154.6	116.4	325.0 — 463.2	394.1
Female	6	194.4 — 326.8	260.6	235.0 — 389.0	312.0
Male	5	151.4 — 203.2	177.3	207.9 — 239.7	223.8
		Triatoma sordida			
1st	74	0.625 — 0.645	0.63	0.748 — 0.868	0.81
2nd	10	2.30 — 2.72	2.5	6.7 — 9.2	7.9
3rd	18	6.1 — 7.7	6.9	17.6 — 25.4	21.5
4th	10	14.4 — 16 6	15.5	66.3 — 86.0	76.1
5th	9	51.6 — 68.1	59.9	199 1 — 274.3	236.7
Female	7	87.2 — 144.9	116.0	46.9 — 101.5	74.2
Male	9	88.5 — 111.4	100.0	33.1 — 55.1	44.1
		Triatoma brasiliensis			
1st	39	1.725 — 1.775	1.75	8.1 — 8.7	8.42
2nd	9	4.4 — 6.7	5 6	27.3 — 33.9	30.6
3rd	9	12.1 — 15.2	13.7	45.2 — 66.4	55.8
4th	9	27.4 — 44.6	36.0	118.9 — 210.7	164.8
5th	8	93.1 — 115 3	104.2	440.6 — 695.3	568.0
Female	6	216.0 — 259.8	237.9	137.6 — 237.8	187.7
Male	5	165.1 — 235.5	200.3	58.2 — 106.0	82.1
		Triatoma maculata			
1st	20	0.246 — 0.283	0.26	0.427 — 0.503	0.46
2nd	12	1.34 — 1.86	1.60	9.9 — 12.8	11.3
3rd	10	4.5 — 6.0	5.3	16.3 — 24.2	20.3
4th	8	12.3 — 15.6	14.0	37.2 — 60.0	48.6
5th	11	32.1 — 41 2	36.7	121.4 — 181.0	151.2
Female	6	109.6 — 148.2	128.9	29.7 — 70.2	50.0
Male	5	96.6 — 124.3	110.5	25.2 — 52.2	38.7
		Panstrongylus megistus			
1st	35	1.138 — 1.184	1.16	7.57 — 8.10	7.84
2nd	12	4.0 — 4.6	4.3	28 5 — 37.3	32.9
3rd	10	12.3 — 17.8	15.1	61.3 — 103.9	82.6
4th	12	45.9 — 60.7	53.3	277.0 — 410.5	343.8
5th	12	126.7 — 196.8	161.7	676.9 — 952.5	814.7
Female	5	284.3 — 419.1	351.7	265.8 — 535.6	400.7
Male	6	212.8 — 286.4	249.6	175.1 — 328.5	251.8
		Rhodnius neglectus			
1st	50	0.260 — 0.272	0.27	1.652 — 1.771	1.71
2nd	10	1.78 — 2.26	2.02	7.5 — 9.8	8.7
3rd	10	3.5 — 4.2	3.9	11.4 — 15.7	13.5
4th	10	10.2 — 12.4	11.3	11.0 — 24.6	17.8
5th	11	23.7 — 29.4	26.6	96.2 — 152.6	124.4
Female	5	55.1 — 74.3	64.7	91.5 — 109.5	100.5
Male	5	47.9 — 69.0	58.5	52.6 — 82.5	67.5

* — Body weight determined in groups of new-born specimens within seven days after emergence and in single older individuals within seven days after molting

** — Blood uptake determined by substracting weight prior to feeding from weight determined immediately after feeding on first meal offered

$\overline{Y}$ — Sample mean

Confidence limits set employing Student's t-distribution

L_1 — lower limit, L_2 — upper limit

The capability of feeding in each stage was determined by testing specimens which had been starved for seven days after molting. At the end of this period the insects were weighed and thereafter allowed to feed undisturbed for one hour before reweighing. Average weights were estimated by weighing of groups of 5-6 newly born insects and from individual weighings in older bugs. Weight was determined to the nearest 0.1 mg on a Mettler-balance.

Results summarized in Table II indicate that there is a continuous increase of blood uptake in the successive nymphal stages followed by a sharp decrease in adults particularly in males.

Insects of various species show the same pattern of changes with regard to blood uptake, thus indicating that the continuous increase of meal size is stage dependent. Another indication that the developmental stage is actively involved in the amount of blood sucked is the observation that emerging adults took up less blood than nymphs from which they emerged, probably due to the fact that adults in ultimate stage of development reached its end at shedding of the exuviae by the 5th instar insects. With regard to the greater amount of blood taken up by female adults, we will proceed on the assumption[5, 14] that this might be associated with egg production.

When results are plotted so that changes in body weight after feeding are demonstrated, the pattern of the feeding strategy becomes of particular interest; it points out in many instances, the very young specimens as the major feeders. For example, 2nd instar nymphs of *T. maculata* and *P. megistus* exhibit an 8-9 fold increase in weight compared to the prefeeding weight, while adults of these two species revealed a 2-2.5 fold increase, at most. However, as can be seen from results, this relationship differs in various species, thus in others no such striking differences were noted (Fig. 2).

Of the six vector species herein tested, *P. megistus* was found to be the most voracious. In the *Triatoma* genus, blood uptake was highest in *infestans* and *brasiliensis*, moderate in *sordida* and lowest in *maculata*.

Although the magnitude of blood meals within species is associated with bug size, as based on body weight, such correlation does not exist between species. For example, the prefeeding mean weight of lst instar in *T. maculata* and *R. neglectus* is practically the same in both species (Table II), yet the blood uptake was 0.46 mg in the former and 1.71 mg in the latter species.

Survival of vector species under starving conditions — This section attempts to outline what is observed about the capability of the bugs to withstand adverse conditions created by lack of food.

Insects utilized in these experiments were derived from laboratory stocks, each representing an individual developmental stage of a given species. These were allowed to feed, utilizing a schedule similar to that which is standardly followed in our laboratory colonies. Insects in each stock were inspected daily for the presence of molted specimens, distinguished easily by their pinkish color. These were removed from the stock, counted, introduced into beakers lined with filter paper and left unfed until death.

Results summarized in Table III indicate that there is almost a progressive increase in surviving capability under starving conditions until the 4th developmental stage. In the 5th stage survival period decreased in three species, insignificantly increased in two others, but continued to decrease in adults.

The most striking fact to emerge from these results is that in certain species unfed adults do not live nearly as long as lst instar nymphs. For example, 4th instar of *T. sordida*, never fed in this stage, lives as long as 213 $\pm$ 4.83 days, female adults, under similar conditions, live 36.4 $\pm$ 1.17 days vs. 47.9 $\pm$ 1.39 days survival by lst instar nymphs.

Such variations, however, were not seen in *T. brasiliensis*, species in which survival capability in successive nymphal stages oscilated around the level reached in the 2nd stage.

The previous experiments dealt with survival capability under complete lack of food. The following were designed to elicit information about the effect of a single blood

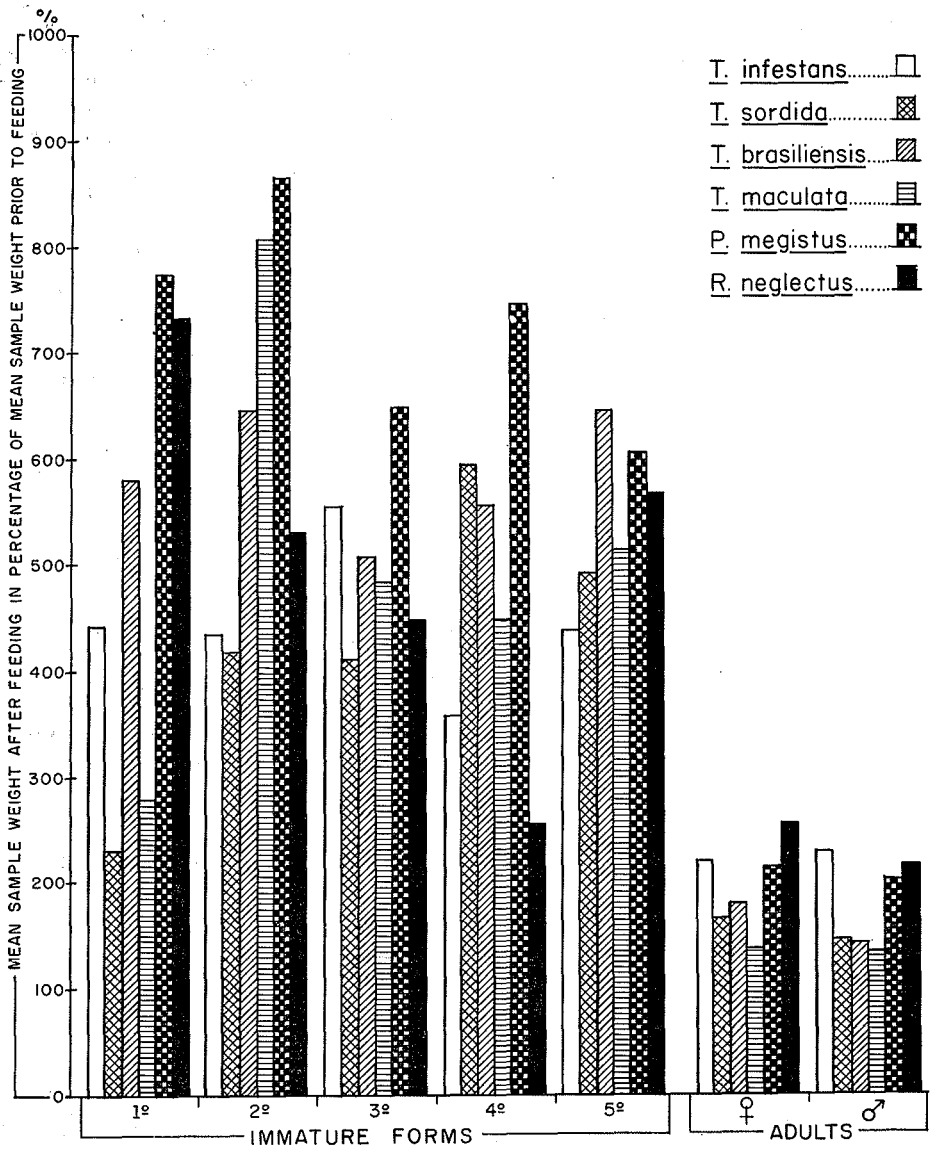

Fig. 2 — Weight of insects after their first meal

meal on longevity of bugs, a determinant of major importance in the xenodiagnostic procedures. In addition information on the extent to which a single blood meal, taken up in one stage, affects the molting process to the next stage was sought.

Three species were utilized in this experiment, two of primary importance as vectors and also routinely used in xenodiagnostic tests in Brazil (*T. infestans* and *P. megistus*) and a third species, *T. sordida* of secondary importance as a vector, so far. The insects were fed after they had been starved for one week in the new developmental stage and thereafter were left undisturbed until all died.

Results recorded in the lower section of Table III show that there occurred a sharp increase in survival capability of lst instar

TABLE III

Survival of immature and mature forms of vectors under starving conditions

Species	Longevity of nymphs in successive stages (days)					Longevity of adults (days)	
	1st	2nd	3rd	4th	5th	female	male
	Means and their standard errors for unfed specimens						
T. infestans	89.0 ± 3.01(30)	146.6 ± 9.91(30)	167.2 ± 9.17(33)	174.5 ± 7.71(44)	127.4 ± 4.77(22)	60.1 ± 2.58(23)	55.2 ± 2.55(14)
T. sordida	47.9 ± 1.39(30)	166.1 ± 2.26(30)	139.9 ± 7.03(28)	213.7 ± 4.83(30)	121.5 ± 13.18(20)	36.4 ± 1.17(25)	47.4 ± 2.00(14)
T. brasiliensis	58.7 ± 1.10(49)	84.7 ± 3.43(44)	76.9 ± 3.64(36)	91.9 ± 6.34(21)	73.1 ± 5.74(25)	86.6 ± 4.08(10)	107.0 ± 4.34(10)
T. maculata	35.3 ± 1.00(50)	32.0 ± 2.20(19)	69.0 ± 3.90(31)	104.7 ± 3.36(25)	122.6 ± 6.77(32)	35.8 ± 1.35(14)	45.4 ± 2.49(9)
P. megistus	35.4 ± 1.77(35)	38.7 ± 2.87(24)	86.4 ± 4.83(24)	98.7 ± 4.82(15)	99.2 ± 2.96(12)	57.5 ± 3.20(10)	65.6 ± 3.34(10)
R. neglectus	13.0 ± 0.92(50)	18.7 ± 0.42(28)	28.7 ± 1.14(25)	65.0 ± 3.51(29)	77.8 ± 2.56(30)	37.2 ± 2.59(11)	38.1 ± 2.39(13)
	Means and their standard errors for specimens fed once						
T. infestans	153.5 ± 4.07(50)	109.2 ± 3.56(35)	130.8 ± 7.10(35)	138.8 ± 5.18(16)	90.4 ± 12.63(20)	66.0 ± 4.21(12)	57.0 ± 7.37(11)
T. sordida	97.7 ± 3.02(31)	128.3 ± 7.18(26)	207.8 ± 4.33(24)	215.4 ± 6.55(20)	158.4 ± 19.87(25)	61.2 ± 2.18(16)	55.2 ± 0.87(13)
P. megistus	82.2 ± 2.98(40)	101.3 ± 5.14(48)	172.6 ± 5.18(20)	210.7 ± 8.37(20)	116.2 ± 10.54(23)	44.5 ± 2.68(13)	66.5 ± 4.50(12)

Numbers in parenthesis indicate sample size

TABLE IV

Frequency of transition from one developmental stage to another in populations fed once in each stage and then starved

Species	Developmental stages									
	1st		2nd		3rd		4th		5th	
	Percent molted									
T. infestans	80	(50)	14	(35)	77	(35)	87	(16)	90	(20)
T. sordida	22	(31)	0	(26)	70	(24)	35	(20)	48	(25)
P. megistus	25	(40)	92	(50)	100	(20)	90	(20)	78	(23)

Numbers in parenthesis indicate sample size

nymphs as compared with that of completely unfed insects. The former exhibits a 2-fold increase in longevity. Changes of a similar magnitude occurred also in the following three developmental stages of *P. megistus*. In the other two species, no such differences were noted between insects fed once only and unfed.

When the single blood meal is analyzed with respect to its effect on the development of the insect, it may be taken as providing the driving force for molting from one stage to another (Table IV).

It is useful to recall that, although insects can survive long periods under starving conditions, they do not undergo development unless blood in an amount appropriate to the developmental stage is taken up (4). But, a single meal may represent far less than needed, thus no molting occurred in the 2nd instar nymphs of *T. sordida*, while 92% of *P. megistus* passed to the third stage under similar conditions.

An analysis of results recorded in Table IV indicates that with the exception of the lst instar nymphs in *P. megistus* and 2nd instar in *T. infestans*, the majority of insects in the remaining stages undergo development, as based on molting to another stage, upon a single exposure to food. Results also suggest that the blood uptake, in a single meal by *T. sordida*, represents an amount far less than needed for development. Molting, in a degree comparable to that in the other species, occurred only in the 3rd stage of *T. sordida*.

DISCUSSION

The fact that vector species differ in their first response to external food supply indicates the presence of inheritance variations arising from differences in the genetic constitution of individuals within a population or between populations of different species. This is a normal expectation and could occur in any insect species. However, the striking fact that emerges from this observation is the influence of this determinant on the generation period, as based on the length of time from hatching to adult development.

Evidence is available from previous studies by the Author [13] that in a group of newly born *T. infestans*, all well engorged on the 4th day after hatching, adult emergence started as early as on day 67, while in another group composed of nymphs chosen at random, with regard to feeding [14], adults started to appear as late as on day 87.

Experiments to be published, which dealt with interspecific differences in generation periods, demonstrated that the length of time from hatching to first adult emergence was shortest in *T. sordida* and longest in *T. bra-*

siliensis. The other species in descending order were *T. infestans*, *P. megistus* and *T. maculata*. So far, *R. neglectus* has not been investigated in this aspect.

This pattern of earliest adult emergence in various species has a provocative similarity with the pattern of interspecific feeding behaviour of newly hatched insects, thus suggesting a behavioural interaction between feeding capability and generation time. It clearly points out the early feeding capability as a major contributor to fast adult emergence.

The demonstration that blood uptake is stage and species dependent is not novel. That blood uptake increases progressively throughout the successive developmental stages reaching a peak in the 5th instar nymphs, followed by an abrupt fall in adults, has been shown previously by several investigators and by us[5, 7, 14].

Although laboratory colonies are fairly homogeneous, strains of insects utilized in determination of biological parameters are variable, lacking genetic uniformity, thus indicating that significance in comparing determinants reported by different investigators is of reduced value. For example, the strain of *T. infestans* derived from the state of Minas Gerais and studied by us previously[14] differs considerably from that herein studied with regard to body weight and blood uptake. Furthermore, continuous rearing of insects under laboratory conditions changes parameters of biological characteristics, as is demonstrated in the following example. The sample mean of body weight and of blood uptake in the first laboratory generation of *P. megistus*, herein described, was for the 5th instar nymphs, 161.7 $\pm$ 15.94 and 814.7 $\pm$ 62.6 mg respectively. Experiments in progress revealed a striking decrease of these values after three years of colonization in the laboratory. The sample means of body weight and of blood uptake in 5th instar nymphs were found to be 77.8 $\pm$ 3.89 mg and 330.5 $\pm$ 28.1 mg respectively.

Such changes, however, were not noted in the relationship between post and prefeeding body weight, which seems to be preserved, exhibiting relatively minor differences. Whether this characteristic can be used in standardization of strains remains to be seen.

While the study of vector feeding strategy is rewarding in its own right, the opportunities presented by information gained with regard to other investigations cannot be overlooked. Interest in the feeding behaviour is increased by the hypothesis raised that susceptilibity of bugs to infection may be stage dependent. Although this is only a hypothesis, we mentioned it, because investigators are in agreement that *T. cruzi* causes more severe infection in young persons than in adults and young animals likewise suffer more intense infection than older individuals[3, 9].

In general investigators are concerned with invertebrate host specificity, the criterion of which is the ability of the parasite to transmit infection through the invertebrate host. In xenodiagnosis, however, attention should be focussed on host susceptibility, characterized by the criterion of capability of the parasite to infect the invertebrate host.

It has also been tacitly assumed that the carrying capacity for the flagellate is elevated by ample blood uptake, thus prompting investigators to utilize 4th or 5th instar nymphs in xenodiagnostic procedures. Currently 3rd instar nymphs are also utilized in tests[10, 19].

Inasmuch as the volume of blood and host specificity is important, host susceptibility cannot be overlooked. BRENER[1] states, "that in some instances triatoma bugs proved to be unsusceptible to *T. cruzi* infection even after ingestion of large numbers of blood parasites from human and animal infection".

If susceptibility to infection in the invertebrate host follows the pattern seen in the vertebrate host, the utilization of young immature specimens may prove more suitable with regard to the sensitivity of xenodiagnostic procedures.

Small volumes of blood taken up by the very young insects, although narrowing the carrying capacity for the flagellate, could be counterbalanced by the greater susceptibility, if present, and also by increasing the number of insects per test. Such a technique would limit the practicability of the test, because examination of great numbers of tiny insects for the presence of flagellates would be rather tedious, but it has the advantage of being performable in the xenodiagnostic procedures simplified by MACKELT[11], in which insects

that had fed on patients were homogenized and the sediment examined for the presence of flagellates.

It is at present impossible to predict the outcome of such studies, but that they are performable is demonstrated by the fact that young developmental stages are capable to ingest such considerable amounts of blood.

Xenodiagnosis is done by feeding of bugs on patients and thereafter the insects are examined for the presence of flagellates. This involves examination of bug feces for characteristic forms of *T. cruzi* 30 days after the feeding on patients. Currently, bugs are examined as late as 60 and 90 days after exposure to infection [10, 18, 19].

As shown by results summarized in Table IV, a single blood meal would allow the majority of nymphal *P. megistus* and *T. infestans* to molt, thus raising the question as to whether parasites consumed by one stage survive through transformation of the host to the next stage, or if infection is limited to the developmental stage in which it is acquired.

After feeding on patients, bugs were routinely given "booster" meals on animals refractory to Chagas Disease. Recently, bugs are maintained under starving conditions during the period from feeding on patients until examination for the presence of flagellates in the feces [19].

Results obtained in these experiments and in others [12, 14, 16, 17] indicate that bugs could remain alive for long periods of time upon a single blood meal, thus suggesting that "booster" meals, as applied by many investigators, are not necessary.

This, however, leaves some important questions unsettled namely [1], does the life of the flagellate approach the life expectancy of the transport host under starvation [2], does the life cycle of the parasite, expressed in a sequence of irreversible morphological transformations, continue independently of external food supply of the host and [3], do "booster" meals allowed on succeeding occasions suppress the multiplication of flagellates in the midgut due to an antiparasitic effect in the blood of the refractory animals utilized for feeding of bugs.

It was also demonstrated that sex of insects affects the meal size, thus female adults were found to take up considerably greater amounts of blood than male adults. This has been stated previously by us [14] and by others [5, 7].

Another indication that the sex of the insect influences the quantity of blood consumed is a recent observation of the feeding behaviour of 5th instar nymphs in *P. megistus*, colonized in our laboratory since 1969. The average meal size was 387 mg in female nymphs and 279 mg in male nymphs. There was notably less difference between the weight of the two sexes than between weight of blood uptake.

Besides the effect of sex on blood uptake, sex may also work toward elevation of host susceptibility to infection. HAUSCHKA [8] states that symptoms of Chagas Disease appeared more severe in male than in female mice during the course of routine subinoculation of *T. cruzi;* for 1568 flagellates in males there were 912 in the females, per 100 microscopic fields.

This is certainly not to say that what occurs in the mammalian host will also occur in the invertebrate host. Nonetheless, information accumulated from studies on the vertebrate-parasite relationship underlines the necessity for similar studies on the insect-parasite relationship, knowledge of which may contribute to the elevation of the sensivity of xenodiagnostic techniques.

RESUMO

Interação dos fatores "espécie" e "instar" no comportamento de vectores da doença de Chagas em relação ao repasto sanguíneo (especulações sobre a importância de determinantes na padronização e na eficácia dos métodos xenodiagnósticos).

Na hipótese de que os conhecimentos relativos ao comportamento alimentar do vector da doença de Chagas, possam servir na elaboração de métodos xenodiagnóstico tão sensíveis na fase crônica como o tem sido na fase aguda da doença, relatamos, no presente trabalho, observações sobre a estratégia alimentar nas seis espécies do vector.

Detivemo-nos, em particular, sobre: 1) Comportamento do inseto recém-eclodido diante do primeiro repasto sanguíneo; 2) Hematofagismo em todas as fases evolutivas do vector, a partir do recém-nascido até a eclosão da imago; 3) A resistência à completa falta de alimentação e 4) A sobrevivência e a capacidade de evoluir após um único repasto sanguíneo.

Os dados obtidos sobre a interação de fatores "espécie" e "instar" nos hábitos alimentares, permitem especular sobre a sua possível utilização na elaboração de métodos xenodiagnósticos.

A demonstração de que formas bem jovens são capazes de ingerir consideráveis quantidades de sangue e de que o inseto fêmea suga mais sangue do que o inseto macho, indica que se deva dar atenção a esses determinantes, quando se procura eleger um inseto modelo para o xenodiagnóstico.

Nossa opinião é baseada na hipótese de que a idade e o sexo do inseto possam participar na suscetibilidade à infecção com *T. cruzi*, do mesmo modo que participam na suscetibilidade à infecção nos mamíferos. Isso, todavia, não significa que o que ocorre no vertebrado, necessariamente ocorrerá no invertebrado. A plausibilidade dessa hipótese, porém, deve ser verificada através de estudos sobre a associação inseto-flagelado.

O fato de que os vectores da doença de Chagas, alimentados uma única vez, possam viver por longos períodos, indica que os repastos de reforço permitidos em ocasiões sucessivas, após alimentados em pacientes chagásicos, não são necessários. Entretanto, levantam-se as seguintes questões: 1) Se a longevidade do *T. cruzi* se aproxima da do hospedeiro invertebrado, sob condições de jejum e 2) Se a seqüência de transformações morfológicas irreversíveis que o flagelado sofre no estômago do hemíptero é independente do suprimento alimentar externo.

A demonstração de que uma quantidade adequada de sangue, em um único repasto, condiciona a muda de um instar para outro, sugere que, pelo menos, uma fração de insetos, quando alimentados em chagásicos, sofrerá transformação para o próximo instar. Isto levanta as seguintes questões: 1) Se a infecção no hospedeiro invertebrado é limitada ao instar em que é adquirida e 2) Se os parasitas ingeridos por determinado ínstar sobrevivem às transformações para o ínstar subseqüente.

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