filled box under stereomicroscopic control. The samples were homogenized in 0.15 ml of cool 0.37 M trichloroacetic acid (TCA) and centrifuged for 15 min at 12,000 × g. The sediment was resuspended in 0.5 ml of 1 M NaOH and stored at 4 °C, while the supernatant was immediately frozen and stored at −80 °C. Protein was assayed in 0.1 ml aliquots of resuspended sediment by means of the spectrophotometric method of Lowry et al.[12]. Cyclic AMP was determined in 0.03 ml aliquots of the supernatant by a competitive protein binding technique, using a cyclic AMP assay kit (The Radiochemical Center, Amersham). The theoretical curve fitting the experimental data was calculated by means of multiple regression analysis according to the Cosinor procedure[13]. Cosine functions may be fitted to a time series by different procedures. In this case, the amplitude and acrophase of the cosine function were estimated by linear regression techniques (least squares method) applied to the data after their transformation by sine and cosine functions. Data need not necessarily be equally-spaced over each cycle of the rhythm investigated. To test the null hypothesis F statistic was used according to the quoted procedure.

*Results.* The mean cyclic AMP concentration in the preoptic region was lower in the L period than in the D period. The analysis of mean hourly values showed that minimum L and maximum D levels were attained through continuous changes according to a synusoidal function (table).

Hourly cyclic AMP fluctuations were also observed in the parietal cortex. However, the existence of a significant daily rhythm was not supported by statistical analysis (table). Such a result may depend on the averaging of small out-of-phase fluctuations of cyclic AMP in single animals. The difference with respect to the preoptic region is noteworthy even in this case.

The results show the existence of a daily rhythm of cyclic AMP concentration in the preoptic region. However, the small amplitude of such a rhythm does not warrant at present any inference on its functional significance. Nevertheless, in view of cyclic AMP involvement in central synaptic events[14], the preoptic daily rhythm may be considered as the result of fluctuations in the activity of hypothalamic and brain stem neurotransmitters influencing the nucleotide's synthesis[15,16]. Experimental findings in the cat[17], mouse[18], and rat[19,20], support this hypothesis.

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2 To whom reprint requests should be addressed.
3 N.J. Perlow, B. Festoff, E.K. Gordon, M.N. Ebert, D.K. Johnson and T.N. Chase, Brain Res. *126*, 391 (1977).
4 J.B. Katz, G. Valases, G.N. Catravas and S. Wright, Jr, Life Sci. *22*, 445 (1978).
5 P.P. Choma, S.K. Puri and R. Volicer, Pharmacology *19*, 307 (1979).
6 M. Mikuni, Y. Saito, T. Koyama and I. Yamashita, J. Neurochem. *36*, 1295 (1981).
7 B. Rusak and L. Zucher, A. Rev. Psychol. *26*, 137 (1975).
8 R.Y. Moore and V.B. Echler, Psychoneuroendocrinology *1*, 265 (1976).
9 A.A. Borbély, Progr. Neurobiol. *10*, 1 (1978).
10 M. Menaker, J.S. Takahashi and A. Eskin, A. Rev. Physiol. *40*, 501 (1978).
11 M. Palkovits, Int. Rev. Cytol. *56*, 315 (1979).
12 O.H. Lowry, N.A. Rosebrough, A.L. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
13 F. Halberg, E.A. Johnson, W. Nelson, W. Runge and R. Sothern, Physiol. Teach. *1*, 1 (1972).
14 J. Daly, Cyclic nucleotides in the nervous system. Plenum Press, New York 1977.
15 M. Palkovits, Acta morph. hung. *26*, 211 (1978).
16 M. Palkovits, L. Záborsky, A. Feminger, É. Mezey, M.I.K. Fekete, J.P. Herman, B. Kanyicsa and D. Szabó, Brain Res. *191*, 161 (1980).
17 D.J. Reis and R.J. Wurtman, Life Sci. *7*, 91 (1968).
18 J.P. Natali, A. McRae-Degueurce, P. Keane, G. Debilly and J.F. Pujol, Brain Res. *191*, 205 (1980).
19 J. Manshardt and R.J. Wurtman, Nature *217*, 574 (1968).
20 I.M.M. Kwilecki and G.D. Prell, Pharmac. biochem. Behav. *12*, 549 (1980).

## Protein level in the haemolymph of the wasp *Polistes gallicus* L. at the beginning of imaginal life and during overwintering. Action of the strepsiterian parasite *Xenos vesparum* Rossi

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*Summary.* During the imaginal life of male *Polistes* wasps, the protein concentration in the haemolymph remained constant. In females, there were 2 groups; one in which this concentration was also stable and another in which it increased. No difference was detected between the haemolymphatic protein level of stylopized males and normal ones. All parasitized females exhibited low haemolymph protein levels similar to those of the low level group.

The physiology of *Polistes* wasps is greatly affected by the presence of the parasite *Xenos vesparum* Rossi (Strepsiptera). One of the most drastic effects of this stylopisation is parasitic castration resulting in the suppression of normal ovarian development in adult females. Modifications occur both in the corpora allata and the neurosecretory cells of the pars intercerebralis[1-6].

Previous works indicate that a large increase in the size of the *Xenos* larvae takes place during the first 15 days of the imaginal life of parasitized wasps. At this time, the endoparasitic larvae feed on the host haemolymph without causing any tissue damage[6,7]. Several authors have pointed out that in some insects, parasitic castration is accompanied by a depletion of haemolymphatic protein[8-11]. Thus it seemed of interest to study the variations of haemolymph protein levels during the imaginal life of the wasp *Polistes gallicus*.

*Material and methods.* The wasps were reared in the laboratory under standard conditions[5]. For studies carried out

during the months of July and August we used 1-25-day-old wasps born in the laboratory. Parasitized wasps were obtained by experimental infestation which entailed placing triongulinid *Xenos* larvae on the young wasps larvae. Since *Xenos vesparum* is strictly endoparasitic up to the 25th day of the imaginal life of *Polistes*, the presence of the parasite had to be checked by dissection. During autumn, we used specimens either collected from the natural environment or born in the laboratory. The overwintering females were collected in the wild during the months of December and January and kept in a cold room at 11 °C.

In order to study haemolymph protein, the head was cut off and a drop of warm wax was applied to seal the wound and thus to prevent the digestive tract from emptying. With a razor blade, a piece of dorsal cuticle was gently excised, then the animals were centrifuged for 5 min at 2000 rpm and 4 °C. 1 or 2 µl of haemolymph were collected from each animal and total proteins were determined by the method of Lowry et al.[12].

*Results.* In the table we have summarized the mean values obtained for each group studied. In summer, no difference could be found between the haemolymphatic protein levels in normal males, parasitized males and parasitized females. For these 3 groups, the values observed were quite similar and did not exceed 20.7±3.2 mg/ml. The haemolymph protein concentration in normal females was significantly higher (31.7±4.7 mg/ml). Such a discrepancy between normal and parasitized females could be observed during autumn and in overwintering females. A more detailed study did, however, uncover some differences at the beginning of imaginal life (fig. 1). During the first 10 days, protein concentration did not significantly differ in normal and parasitized females. In the 15 days thereafter, it rose markedly in one group of normal females and became significantly higher than in parasitized animals. In the males, no such difference was observed (fig. 2); protein concentration was similar in normal and parasitized animals and remained low throughout imaginal life.

Another noteworthy finding was the way in which individual values for normal females followed different patterns (fig. 1). In the first 5 days, the values obtained were rather homogeneous but a noticeable scattering of the points began thereafter. Thus the unimodal distribution observed at the onset of imaginal life gradually gives way to a quite bimodal distribution.

It therefore seems that females may be divided into 2 groups with high or low haemolymph protein concentration, respectively. These differences exist from the 10th to 25th days of imaginal life and on into the autumn group. This group is in fact made up of older females whose age is not precisely known. If some of these wasps belong to the worker caste, they must die during winter. In the group collected in natural overwintering conditions, the protein level is fairly high. In fact, of the 2 populations found in normal females, one maintained a constant low protein level up to the winter time. There is no difference between this low level population and the parasitized group.

*Discussion.* Our data concerned varations in protein titre in the haemolymph of *Polistes gallicus* at the beginning of imaginal life. As in other species, such as *Pyrrhocoris apterus*[13] and *Anacridium aegyptium*[10], males where observed to have a low protein level throughout their reproductive period (they die at the end of summer). In *Bombus terrestris* females[9] and in worker honeybees[14] protein titre increased during the beginning of imaginal life. A similar increase was observed in part of the normal female population of *Polistes*.

Protein concentration in haemolymph during imaginal life of normal and parasitized wasp *Polistes gallicus*

| | | Number of animals | Mean ± SEM |
|---|---|---|---|
| Summer | Normal males 1-25 days old | 44 | 16.5 ± 1.8* |
| | Parasitized males 1-25 days old | 42 | 16.6 ± 2* |
| | Normal females 1-25 days old | 59 | 31.7 ± 4.7 |
| | Parasitized females 1-25 days old | 38 | 20.7 ± 3.2* |
| Autumn | Normal females | 31 | 33.7 ± 7 |
| | Parasitized females | 7 | 14.8 ± 7* |
| Winter | Normal females | 23 | 50.7 ± 5.6 |
| | Parasitized females | 6 | 21 ± 2.3* |

* Significantly less than normal corresponding females values at $p < 0.01$.

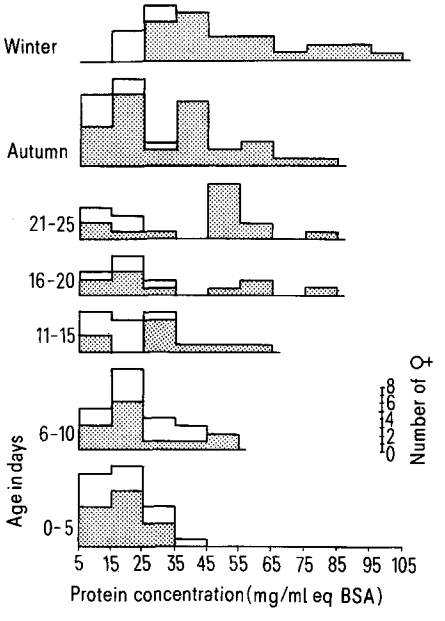


Figure 1. Evolution of haemolymph protein concentration during imaginal life of normal and parasitized females. ▨, normal females; □, parasitized females.

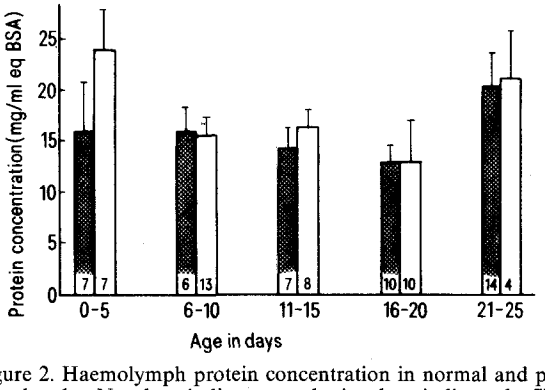


Figure 2. Haemolymph protein concentration in normal and parasitized males. Numbers indicate sample size; bars indicate the SEM. ▨, normal males; □, parasitized males.

We must underline that parasite development occurs in larvae and pupae of both sexes. The parasite grows greatly by feeding on its host so that the ovaries of newly-emerged parasitized females are smaller than those of normal newly-emerged females. The endocrine interactions necessary for the host's metamorphosis are however preserved. The most obvious effects of parasitization on endocrine organs were observed in the imaginal population[2-6].

Infestation by *Xenos vesparum* has different effects on the levels of protein measured in the haemolymph of male and female *Polistes* wasps. As when *Anacridium aegyptium* is parasitized by a fly[10], there is no apparent difference between the protein levels of normal and parasitized males. As shown in figure 1, up to the 10th day of imaginal life, the haemolymph protein level in normal and parasitized females was similar. It is known from previous work that during the first 15 days of a wasp's imaginal life, *Xenos* undergoes a great increase in size with particularly rapid development of its fat body; this intense metabolic activity at this point in the life of the parasite coincides with its prenymphal stage[6,7]. In spite of the growth requirements of the parasite at this time, the haemolymphatic protein level is not significantly, lowered.

Thus it is clear that during the main feeding period of the parasite's life, which takes place in the pre-imaginal and the early beginning of imaginal life of the wasp, the physiology of the host serves the parasite's requirements. In fact, at this point, it is difficult to find important differences between normal and parasitized females.

Paradoxically, it is only when the parasite is full grown, has metamorphosed and no longer feeds on the host that the physiology of the host begins to show abnormalities, and we can measure the most significant differences between normal and parasitized female wasps. In the infested wasps, the ovaries, fat body and corpora allata do not develop in such a way as to allow reproduction. This inability is certainly due to the early presence of the parasite, since the removal of the full grown parasite does not restore normal reproductive physiology[4].

The present experiments reveal that the real difference lies in the fact that the haemolymphatic protein level in certain normal wasps rose after the 10th day, while it did not in parasitized females.

The presence of the parasite was seen to prevent the protein titre from increasing, as in some normal females, and caused all parasitized females to have low titres. The haemolymph of the bug *Eurygaster* has been observed to be devoid of vitellogenic proteins and to be deficient in some non vitellogenic proteins[15] when the animal is infested by phasiid flies. This is not the case in *Polistes* since disk electropheresis on polyacrylamide gels revealed a female-specific fraction in both normal and parasitized animals[16]. As in *Bombus terrestris* parasitized by a nematode[9], parasitized *Polistes* females were seen to have a lower concentration of yolk material than non parasitized animals.

In their study of locust parasitized by a nematode, Gordon et al.[8] suggest that the parasite alters the nutritional composition of the haemolymph by stimulating the host fat body to catabolize proteins and to release amino acids into the haemolymph for its consumption. Median neurosecretory cells and the corpora allata are involved in this process. In the case of *Polistes*, a rapid discharge of the pars intercerebralis neurosecretory cells together with a decrease in the size of corpora allata have been observed[6]. In *Anacridium aegyptium* parasitized by a tachinid fly, a hypoactivity of median neurosecretory cells is involved in the parasitic castration[10,17]; the corpora allata are active[18]. In *Polistes*, the corpora allata of stylopized wasps appeared to be inactive since in parasitized females, ovarian development can be stimulated by implantation of active corpora allata[19], since in vitro experiments show that the synthetic activity of the corpora allata is lower in parasitized females than in normal females[20] and since the level of circulating juvenile hormone is very low (Strambi, unpublished observations).

While newly emerged parasitized females are hindered from normal reproduction, they can nevertheless overwinter as well as normal future foundresses. An important factor in this hindrance is the lack of juvenile hormone production.

In *Polistes*, only queens are able to overwinter and found spring nests. There is however no morphological difference between queens and workers to explain this ability. In a study on *Polistes exclamans* Eickwort[21] notes certain qualitative and quantitative differences in the parietal fat body of females and suggests that the fat body of putative future queens is more abundant and that this enables them to hibernate. This postulate seems however to be contradicted in our study since we observed that, even though parasitized *Polistes gallicus* have very thin yellowish fat bodies and their haemolymphatic protein levels are low, they can overwinter as well as normal females. It should nevertheless be recalled that at the beginning of the imaginal life of *Polistes* there is a correlation between haemolymph protein concentration and the size of adipocytes[22]. If indeed the overwintering females have more fat body, it is likely that these reserves are used for maturing the first eggs the following spring. Thus it may be that the bimodal distribution of protein level which we observed in normal *Polistes* females is related to Eickwort's observation and that both findings may be used as physiological criteria in classifying the progeny of *Polistes* as workers or overwintering future foundresses.


1 J. Brandenburg, Z. Morph. Ökol. Tiere *45*, 343 (1956).
2 A. Strambi, C. r. Acad. Sci. *260*, 3768 (1965).
3 A. Strambi, C. r. Acad. Sci. *263*, 533 (1966).
4 A. Strambi, C. r. Acad. Sci. *264*, 2646 (1967).
5 A. Strambi, Doctoral thesis, Univ. Paris, Sci. nat., Paris 1969.
6 A. Strambi and C. Strambi, Archs Anat. microsc. Morph. exp. *62*, 39 (1973).
7 A. Strambi and C. Strambi, C. r. Soc. Biol. *167*, 1645 (1973).
8 R. Gordon, J.M. Webster and T.G. Hislop, Comp. Biochem. Physiol. *46B*, 575 (1973).
9 I. Röseler and P.F. Röseler, J. Insect Physiol. *19*, 1741 (1973).
10 J. Girardie, J. Insect Physiol. *23*, 569 (1977).
11 S.B. Vinson and G.F. Iwantsch, Q. Rev. Biol. *55*, 143 (1980).
12 D.G. Lowry, J. Rosebrough, A. Farr and R.J. Randall, J. biol. Chem. *193*, 265 (1951).
13 K. Slama, J. Insect Physiol. *10*, 773 (1964).
14 W. Rotz, L. Gerig, H. Wille and M. Lüscher, J. Insect Physiol. *22*, 1485 (1976).
15 T.G. Belyaeva, Zool. Zh. *57*, 548 (1978).
16 C. Roux, C. r. Acad. Sci. *276*, 3159 (1973).
17 J. Girardie and A. Girardie, J. Insect Physiol. *23*, 461 (1977).
18 J. Girardie and L. Joly, C. r. Acad. Sci. *281*, 719 (1975).
19 A. Strambi and A. Girardie, C. r. Acad. Sci. *276*, 3319 (1973).
20 P.F. Röseler, I. Röseler and A. Strambi, Insectes Soc. *27*, 97 (1980).
21 K. Eickwort, Insectes Soc. *16*, 67 (1969).
22 M. Pratte, C. Strambi, A.Strambi and J. Gervet, Insectes soc., in press.