

INVESTIGATIONS ON THE *IN VITRO* FEEDING AND *IN VITRO* BREEDING OF THE HUMAN BODY LOUSE *PEDICULUS HUMANUS CORPORIS* (ANOPLURA: PEDICULIDAE)

BIRGIT HABEDANK¹, GABRIELE SCHRADER², STEPHAN SCHEURER³,
AND EBERHARD SCHEIN¹

¹ Institute of Parasitology and Tropical Veterinary Medicine, Faculty of Veterinary Medicine, Freie Universität Berlin, Germany

²Institute for Water-, Soil- and Air-Hygiene, Federal Environmental Agency, Berlin, Germany

³Institute of Tropical Medicine, Berlin, Germany

Abstract - The human body louse *Pediculus humanus corporis* - the main vector of both human spotted fever (rickettsiosis) and relapsing fever (borreliosis) - is maintained for insecticide efficacy tests and is commonly reared on rabbits as substitute hosts. A membrane feeding technique may offer a return to the natural nutrition medium of body lice - human blood - for feeding and reproducing these host specific human parasites. *P. humanus corporis*, derived from a rabbit adapted strain, were offered preserved human blood through a Parafilm M[®] membrane (American National Can, Chicago) at 38 °C. The superimposed blood, stemming from a blood bank, contained a CPD stabilizer. Subsequent to initial experiments on nutrition media and storage condition, *P. humanus corporis* were reared continuously for a nine-month period until adults of the 9th generation emerged. Lice regularly showed a feeding rate of more than 90% and a low mortality rate on day one after nutrition. Engorging time became shorter, from about 90 min (generation 1) to 15-30 min (generation 5), similar to the engorging time of *P. humanus corporis* on rabbits. To conclude, preliminary results indicate that *in vitro* feeding could be an alternative method of breeding the human body louse. Membrane feeding saves laboratory animals and is therefore considered to be a contribution to animal welfare. Further studies are required to optimize the composition of the nutrition medium.

Key words - Lice, alternative rearing, membrane feeding, Parafilm M, porcine blood

INTRODUCTION

The human body louse *Pediculus humanus corporis* de Geer, 1778 poses an important hygiene problem and is of significance as a main vector of zoonoses, such as spotted and relapsing fever caused by *Rickettsia prowazeki* and *Borrelia recurrentis* respectively (Murray and Gaon, 1973; Burgdorfer, 1976; Weyer, 1978). Control of such infections is based on effective louse control. Regular efficacy tests of commonly used as well as new insecticides are required. The source of many tests are laboratory-reared human lice.

Rearing of both the body and head louse had for many years been practicable on humans only (Culpepper, 1944; Lang and Roan, 1974) until the body louse had adapted itself to feeding on the only substitute host – the rabbit (Culpepper, 1948). Rabbits must lie on their backs and have their legs fixed to a special table (for about one hour) because lice are only able to suck blood on the shaved rabbit abdomen. This feeding technique does not correspond to the natural feeding performance and is hardly compatible with animal welfare. There is therefore a need for establishing *in vitro* feeding techniques for maintenance and mass rearing of *P. humanus corporis*.

Fuller *et al.* (1949) and Haddon (1956a, b) fed *P. humanus corporis* through membranes during a 14-day and 48-day period, using chicken skin or Gutta percha membranes (similar to natural rubber) and defibrinated human blood or defibrinated haemolysed human blood respectively. A study by Lauer and Sonenshine (1978) involving defibrinated rabbit blood revealed that the blood uptake by the body louse reached a low level. Mumcuoglu and Galun (1987) observed sucking rates of the body louse after offering different blood fractions and their components through silicone-parafilm membranes. Despite

of these promising feeding studies, a continuous breeding of the human louse in a laboratory using membrane feeding technique, has yet to be established.

The objectives of the presented study were to: 1) confirm the membrane feeding method for *P. humanus corporis* (feeding system, membrane, necessity of olfactory stimulation); 2) evaluate feeding media continuously available for nutrition of a lice colony; and; 3) establish long-term *in vitro* breeding of *P. humanus corporis*.

MATERIALS AND METHODS

Body lice

The body lice for feeding experiments derived from a body louse strain that is maintained for about 30 years at the Institute for Water-, Soil- and Air-Hygiene (IWSA, German: WaBoLu) of the Federal Environmental Agency. Lice were fed on rabbits (races: Chinchilla bastards or Sandylop) 4 to 5 times per week for 15-20 minutes. Between daily feeding times the parasites were stored at 32 °C and 60% relative humidity (RH) and on non-feeding days at 25 °C and 60% RH.

Adult and first larval lice stages were used for *in vitro* feeding experiments. Lice of the same age were at random divided into equal experimental groups. The last feeding of adult lice on rabbits had been about 24 hours before, with first-stage larvae never having been fed. The number of body lice depended on the surplus of the WaBoLu louse breed. After the daily feedings, lice were stored in darkness in an incubator at 31 °C and 70% RH. On days without nutrition, lice were kept at 19-22 °C and about 70-80% RH.

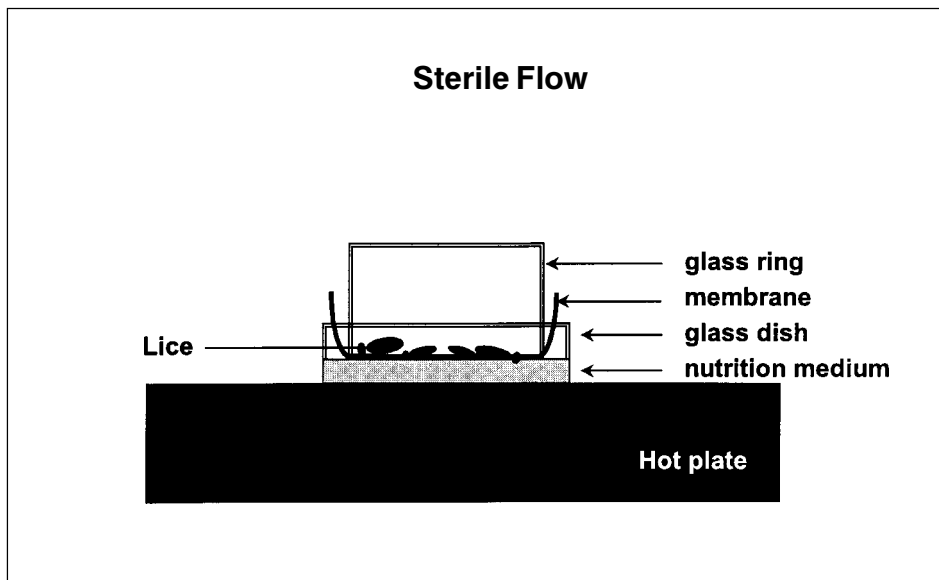


Figure 1. Scheme of the system for artificial membrane feeding of *P. humanus corporis*.

Feeding system. Figure 1 shows the feeding system for *P. humanus corporis*. The devices are similar to those used for feeding the soft tick *Ornithodoros moubata* (Klunker and Kiesow, 1981; Montag et al., 1992; Habedank et al., 1994) or the hard tick *Dermacentor nuttalli* (Habedank and Hiepe, 1993). Commercial Parafilm M® (American National Can, Chicago) was used as a membrane. The figured glass rings were small (d=42 mm, h=40 mm; made by a glass blower for this purpose) to give Parafilm® M more stability when stretched over the ring. Glass dishes had a diameter of 50 mm. The feeding devices were placed onto a hot plate (Stork-Tronic, Präzitherm PZ 28-1, precision 0.1°) in a sterile flow (Gelaire® Flow Laboratories, model HF 48) to prevent nutrition media from being contaminated, thus avoiding the addition of antibiotics or fungicides. A laboratory balance, precision 0.1 mg (Sartorius, Labor Alliance High Tec, LA 140 P), served to determinate louse weight.

Table 1. Sucking rates, weights and mortality rates of adult rabbit-adapted *P. humanus corporis* offered fresh human blood, porcine blood and preserved human blood.

Trial	Feeding medium	Devel. stage	Repet.	n total	m _{hungry} mg ± SD (range)	Sucking rate % ± SD (range)	m _{after nutrition}		Mortality d2 % ± SD (range)
							mg ± SD (range)	% ± SD (range)	
1	Human blood	Ad	4	64	1.45 ± 0.11 (1.29...1.52)	77.8 ± 18.7 (50...88.9)	2.72 ± 0.43 (2.07...2.99)	186 ± 18 (160...201)	4.8 ± 3.2 (0...6.7)
2	Human blood (control)	F	1	39	1.51	94.8	3.33	221	5.1
		M	1	10	0.99	80.0	1.27	128	30.0
	Porcine blood	F	2	78	1.67 (1.65;1.69)	96.2 (94.8;97.4)	2.91 (2.77;3.05)	174 (164;184)	17.9 (10.3;26.5)
		M	2	20	1.10 (1.09;1.10)	75.0 (70;80)	1.63 (1.39;1.86)	148 (128;169)	30.0 (10;30)
3	Human blood (control)	F	1	40	1.14	92.7	2.74	239.6	2.5
		M	1	40	0.92	87.5	1.60	173.9	12.5
	PHB	F	1	40	1.13	100	2.65	234.7	5
		M	1	40	0.93	85.0	1.76	188.2	10
4	Porcine blood	La 1	2	400	0.058 (0.058;0.058)	98.0 (97.5;98.5)	0.107 (0.105;0.108)	185 (182;187)	29.8 (27.0; 32.5)
	PHB	La 1	3	590	0.058±0.003 (0.057...0.061)	96.1± 1.5 (94.5...97.5)	0.144 ± 0.014 (0.133...0.160)	249 ±15 (235...264)	2.1 ± 1.4 (0.5...3.2)

Ad – adult, F – females, M – males, La 1 – first larval stage
m – mean body weight; Mortality d2 – mortality rate one day after feeding
PHB – preserved human whole blood

Table 2. Sucking rates, weights and mortality rates of *P. humanus corporis* fed on PHB on d 1 and d 14 of storage (4 °C vs. -27 °C).

Feeding medium	Devel. stage	Repet.	n total	m _{hungry} mg ± SD (range)	Sucking rate % ± SD (range)	m _{after nutrition}		Mortality d2 % ± SD (range)
						mg ± SD (range)	% ± SD (range)	
PHB d1	F	4	160	1.77 ± 0.04 (1.73...1.81)	98.8 ± 1.4 (97.5...100)	3.97 ± 0.19 (3.80...4.22)	224 ± 7 (217...233)	5.1 ± 2.0 (2.5...7.5)
	M	4	64	1.06 ± 0.03 (1.02...1.09)	87.5 ± 11.4 (75.0...100)	1.55 ± 0.19 (1.28...1.70)	146 ± 14 (126...156)	20.9 ± 5.7 (15.4...26.7)
	La 1	4	800	0.045 ± 0.0005 (0.045...0.046)	84.0 ± 4.2 (79.0...88.0)	0.188 ± 0.005 (0.112...0.122)	260 ± 10 (249...270)	2.0 ± 1.6 (0.5...4.0)
PHB d14 4 °C	F	2	80	1.12 (1.07...1.16)	40.0 (37.5;42.5)	1.70 (1.64;1.76)	152 (152;153)	2.5 (0;5)
	M	2	70	0.82 (0.81;0.82)	51.5 (51.4;51.5)	1.17 (1.13;1.21)	142 (136;148)	10.3 (9.1;11.4)
	La 1	2	400	0.056 (0.055;0.058)	97.8 (97.5;98.0)	0.154 (0.150;0.158)	274 (259;290)	1.8 (0.5;3.0)
PHB d14 -27 °C	F	2	80	1.13 (1.13...1.13)	39.3 (32.5;46.2)	1.53 (1.40;1.66)	136 (125;147)	2.5 (2.5;2.6)
	M	2	70	0.82 (0.81;0.84)	27.9 (27.3;28.6)	1.95 (0.90;1.00)	115 (112;119)	16.1 (15.2;17.1)
	La 1	2	400	0.057 (0.056;0.058)	96.5 (94.5;98.5)	0.149 (0.140;0.158)	263 (241;285)	0.5 (0.5;0.5)

F – females, M – males, La 1 – first larval stage
m – mean body weight; Mortality d2 – mortality rate one day after feeding, PHB – preserved human whole blood

Nutrition media

Fresh human blood was taken from volunteers and stabilized with 1.5-2 IE Sodium-Heparin per ml. The blood was stored at 4°C until use. Porcine blood was taken from the *Vena jugularis* of slaughter animals and treated in the same way. Preserved human blood was obtained from the blood bank of the German Red Cross as three separate units: deep frozen plasma (DFP), erythrocyte concentrate (EC) and buffy coat (BC). The units contained Citrate-Phosphate-Dextrose (CPD)-stabilizer (70 ml per 500 ml blood donation) and EC additional 110 ml Sodiumchloride-Adenine-Dextrose -Mannitol(SADM)-solution. The blood bank stored DFP below -30° C, EC and BC at 4° C. All units were obtained for experimental lice feedings only after transfusion medical expiry date (DFP – 1 year, EC - 40 days, BC is not applied in transfusion medicine). For experimental use, the DFP, EC and BC were reunited into preserved human whole blood (PHB). The PHB was stored per 10-30 ml at 4 °C or -27 °C. For feedings, 2-3 ml of nutrition medium was filled into each dish.

Feeding experiments

The feeding system was restricted to the required minimum: thin membrane (Parafilm® M), nutrition medium, temperature at the hot plate similar to the temperature of the host body (38 °C), no olfactory stimulation, no magnetic blood mixing, day-light. Four samples of adults were exposed to the *in vitro* system for 6 hours. Fresh human blood was offered as optimal nutrition medium (Table 1, trial 1). In the following trials, other promising nutritional sources were examined (exposure 1 to 2 hours). In trials 2 and 3 (Table 1) adult lice were offered porcine blood and preserved whole blood in comparison to fresh human blood controls. The behaviour of larvae in the *in vitro* system was tested in trial 4 (Table 1). First-stage larvae were exposed to porcine blood and preserved whole blood for one hour. This experiment was carried out without fresh human blood control to avoid unnecessary voluntary blood donation. The next step was to establish the nutritive quality of short-time stored PHB. Thus, blood of the same sample was offered to adults and larvae on day 1 of preparation and on day 14 of storage at 4 °C or -27 °C.

Each day, live lice were counted and dead specimens removed. The weight before and after nutrition, measured per lice sample (including lice specimens without blood uptake), served to calculate the mean individual louse weight. Body weight percentage was determined as the quotient of body weight before and after blood uptake. Especially, the mortality rate adds validity to the feeding success. Mortality rate was determined per sample as the quotient of number of died lice at one day after feeding (mortality d2) in relation to the number of lice exposed to the nutrition medium. Data are given as arithmetical mean ± standard deviation (SD). Ranges were noted as (min...max) if three or more repetitions took place and, in case of only two replications, they were given as (value 1; value 2).

To establish a membrane fed colony, *P. humanus corporis* were regularly exposed to superimposed human preserved blood 4 to 7 times per week for 60 to 90 minutes and only in exception (i.e. after storing at 19-22 °C) up to 120 minutes. Colony feeding started with 4 groups of 200 first stage larvae each without previous blood uptake. PHB was used immediately after preparation or after storage 1 to 14 days at 4 °C or at -27 °C. Two samples were offered PHB, stored at 4 °C and two samples PHB, frozen at -27 °C.

RESULTS

During the initial experiment (Table 1, trial 1) the first adults pierced the Parafilm® M membrane only minutes after exposure to the feeding system. Only a low number of adults attached themselves to the membrane at a late stage. In 78 ± 19% of adults, a distinct uptake of freshly obtained human blood was observed. 0 to 6.7% of the lice died within one day after engorgement. When porcine blood was offered to adult body lice (Table 1, trials 2 and 4), 95 to 97% of females and 70 to 80% of males were engorging within one hour. A similar percentage of adults engorged on human blood (control). Moreover, almost all of the exposed first-stage larvae (about 98%) engorged on porcine blood, with most beginning to suck blood immediately after exposure to the membrane. The mortality rate amounted to 18% for females,

30% for males and 30% for the larvae within 24 hours and was noticeable high. Table 1, trials 3 and 4, show the results of PHB uptake. Adult lice revealed similar feeding rates, body mass increases and mortality rates equal to the levels found in adults nourished on human fresh blood (control). 96% of *P. humanus corporis* larvae sucked PHB and reached a body weight percentage of 249% in relation to their hunger weight. But contrary to the porcine blood group, the mortality rate of larvae was low, averaging 2%. These results indicate that PHB was the only medium promising satisfying feeding.

Freshly prepared PHB on day 1 (Table 2) induced a high percentage of blood-sucking adults and larvae, a high increase in mean body weight and caused a low mean mortality rate of 2% in larval samples. When adults and larvae were offered PHB of the same preparation sample after a storage of 14 days, only larvae showed high feeding rates of 94 to 98 %. The high feeding rates occurred independently of the storing temperature (4 °C vs. -27 °C). A small percentage of adults fed on stored PHB (females with a mean of 39 to 40%). However, the mortality rate of female body lice was low (2.5%). It should be noted that a very small volume of blood uptake by adult lice may not be visible to the observer. A low mortality rate of 1.8 and 0.5% was determined for the larvae.

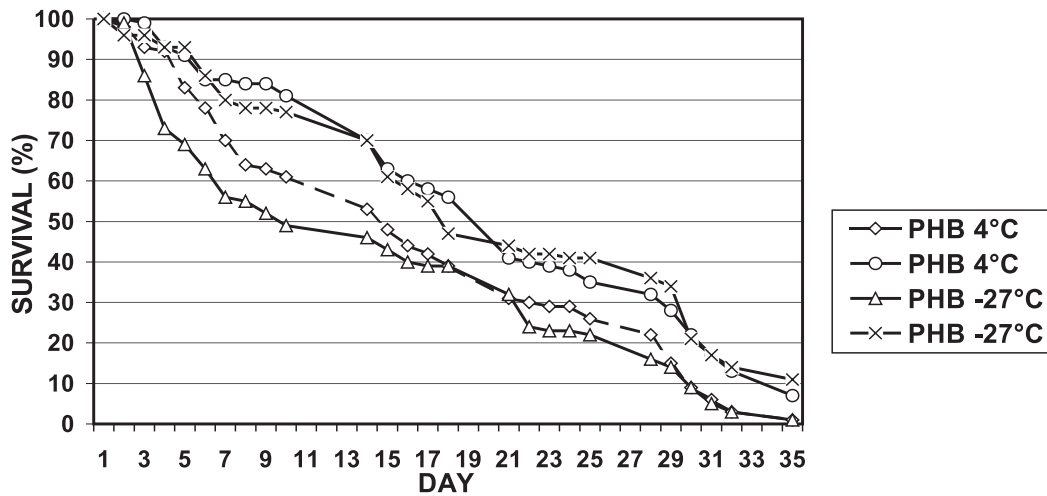


Figure 2. Survival of *P. humanus corporis* after feeding on superimposed PHB stored at 4 °C or -27 °C, generation 1.

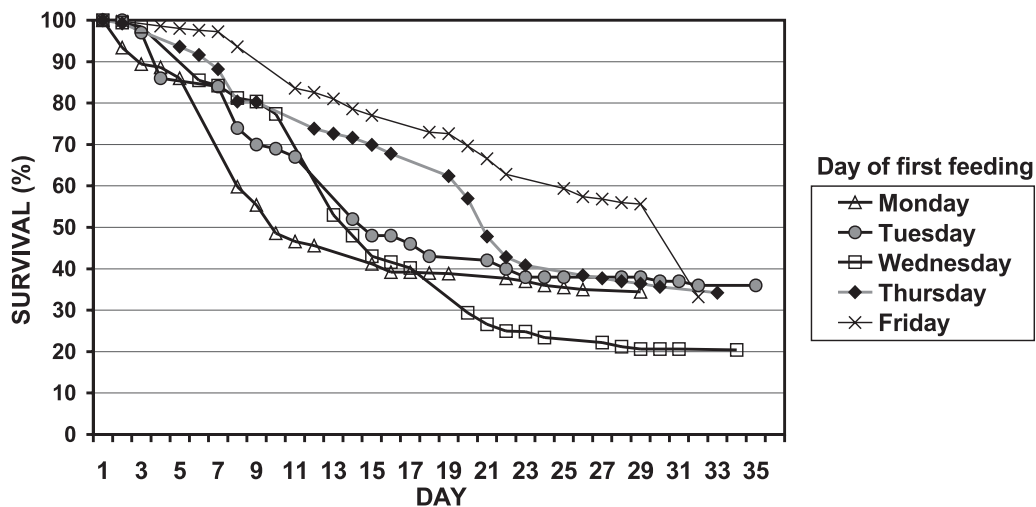


Figure 3. Survival of *P. humanus corporis*, fifth generation, after feeding on superimposed PHB. Feeding started on several days of the week. At weekends (Saturdays and Sundays) lice were without feeding stored at 19-22 °C.

***In vitro* maintenance of *P. humanus corporis*.** 1st generation. The first-stage larvae of *P. humanus corporis* were fed daily for 10 days and after day 10, the feeding intervals were reduced to 4 or 5 days per week. The 35-day survival rates of the four samples are shown in Figure 2. 39-56% of the lice survived 17 days, egg deposition was observed as from day 18 after initial feeding. The number of hatched larvae from deposited eggs, after lice had been fed with PHB stored at 4 °C and -27 °C, was 209 and 427 respectively. Dependent on the nutrition medium storage - and considering four periods without feeding (requiring a lice storage at 19 to 22 °C) - a percentage of 52% and 107% of the larvae, respectively, were reproduced. 2nd-4th generation. Like the parental generation, the second and following generations were fed with PHB (4° vs. -27 °C) as well. Third-generation lice were fed daily to increase the reproduction capacity of the body louse. Starting with the 4th generation (1,060 larvae from the group fed with frozen PHB) nourishing frequency was reduced to 6 days per week. Experiments with the other group fed with PHB, stored at 4 °C, were concluded as they only contained a low number of reproduced larvae. 5th-9th generation. By the fifth generation, more than 500 larvae were hatching per day. The survival rate of larvae (4 samples of 500 larvae and one, Monday-sample containing 240 larvae), resulting from a feeding frequency of five days per week, is shown in Figure 3. Lice were observed to finish their blood meal within 15-30 minutes. The lice regularly showed a feeding rate of more than 90% and a low mortality rate on day after nutrition. The following generations reproduced even more than 500 larvae per day. About 2500 larvae per generation were fed as a reproduction pool. Following daily meals of blood in 3 samples of 40 larvae (7th generation), lice moulted into second-stage larvae within 6-7 days of feeding exposure, into third-stage larvae within 9-11 days, and into adults within 14-16 days. 65 to 83% of lice survived up to day 17 and had reached adult stage. *In vitro* rearing of *P. humanus corporis* lasted until adults of the 9th generation emerged (nine months period).

DISCUSSION

Confirming to investigations by Fuller (1949), Haddon (1956a, b) and Mumcuoglu and Galun (1987), in initial feeding experiment with *P. humanus corporis* was shown that lice can be fed well through membranes. While the authors cited above used defibrinated or citrated human blood as nutrition medium, we added Sodium-Heparin to anticoagulate the fresh human blood. The artificial membrane Parafilm® M may be used for feeding all stages of *P. humanus corporis*. Silicone membranes of low thickness, as used by Habedank and Hiepe (1993) and Kuhnert *et al.* (1995), can be tested as alternative membranes. Attraction of the lice to temperature was sufficient to induce piercing the membranes. Lice tasted and engorged the offered nutrition media.

To summarize, *in vitro* feedings of body lice are possible in a feeding system reduced to a required minimum, not unlike the systems for artificial feeding of soft ticks applied by Howarth and Hakoma (1978), Klunker and Kiesow (1981), Montag *et al.* (1992) and Habedank *et al.* (1994). In addition, we used a sterile flow to avoid contamination of the blood media and to avoid the addition of antibiotics as often practised during *in vitro* feeding of other haematophagic arthropods. After some dead lice with dark or black coloured abdomens had been found, a bacterial or fungal infection had to be assumed. However, repeated microbiologic examinations of the nutrition media showed no evidence of any medium contamination.

Continued *in vitro* maintenance of lice cannot depend on frequent blood donations by human volunteers. Daily lice feedings require a stock of nutrition media. Blood of animals (donor animals or slaughter animals) and units of human blood are continuously available. The pig seemed to be the most promising species among animals. Porcine blood induced high sucking rates in all observed lice stages but caused high mortality rates. In fact, all lice died during a course of repeated feedings within one week (own observations, unpublished). Probably, the host-specific body lice are not able to digest some porcine blood components. A high mortality rate was observed as well by Häfner and Ludwig (1969) in

cases of feeding the pig louse, *Haematopinus suis*. These authors suspect antibiotics in the animal food to be the source of the high mortality. However, porcine blood cannot serve as a nutrition medium for a continuous breeding of human lice.

Preserved human whole blood was tested because units of stored blood are continuously available from the blood bank. The units were used after expiry date only, first to avoid any misunderstanding by blood donors as the experimental use of their blood and, second, to reduce costs for acquiring blood containers. Units of stored whole blood have not been tested for *in vitro* feeding of human lice. When the soft tick *O. moubata* fed on preserved human blood beyond expiry date, it led to a high mortality despite of high feeding rates and high body weight increase (Wirtz and Barthold, 1986; Habedank *et al.*, 1994). As the preservation methods at blood banks have changed substantially during the past years from storing whole blood per transfusion bag to separately storing plasma, erythrocytes and buffy coat, tests of the nutrition efficiency of reunited PHB were required. High sucking rates and high survival rates after blood uptake pointed to the suitability of PHB for nutrition of lice as well as for the permanent maintenance of body lice. Comparing developmental times of PHB-fed lice to rabbit-fed lice under conditions of daily nutrition, it was noticed that they developed into adults within 14...16 days and 12...14 days (own observations, unpublished) after hatching respectively. The difference of about two days may be explained by the dilution of the human blood by the CPD-stabilizer and SADM-solution that cause an additional volume of approximately 36% to the donated human blood. The survival and reproduction of the lice were influenced by the storage temperature and the duration of the period without any feeding. At a temperature of 19-22 °C, a very high percentage of lice survived without a substantial meal by means of metabolic slow-down. On the other hand, these temperatures distinctly increased the mortality rate in larvae during the period of metamorphosis and in females reversibly decreased egg deposition. Machel and Krynski (1976) observed that sexual maturity failed to be reached and eggs failed to hatch when body lice were stored permanently at 20-25 °C. Storage temperature was still not increased considering the dilution of the nutrition medium which caused a lower nutritional value than undiluted rabbit or human blood. Storage confirmed by sufficiently high survival rates and lice reproduction.

To conclude, the PHB offers a return to human blood as natural nutrition medium of the body louse. Despite of increased developmental times, a continuous maintenance of *P. humanus corporis* by membrane feeding on PHB is evidently possible, as demonstrated over 9 generations. These preliminary results indicate that *in vitro* feeding could be an alternative method of breeding and, possibly, mass rearing of the human body louse. Further studies are required to optimize the composition of the PHB. Membrane feeding saves laboratory animals and is considered to be a contribution to animal welfare. Artificial feedings using standardised nutrition media are recommended for medical or biological studies on the vector role of lice, for the examination of the parasite itself and, as already practised by Mumcuoglu *et al.* (1990), for qualitative and quantitative insecticide testing.

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