all the intermediate and small-sized workers pass through a glass tube, slightly smaller than the major's thorax (2.5 mm in diameter). The caste specific difference in appeasement food transfer seems to be due to differential aggressive element present in the largely defensive majors over minors[4].

The regurgitate odour seems to mask the hypothetical alien colony odour[4], which is said to be derived partly from food and nest material[13,14] and partly from genetic make up of the colony[15]. It may itself be regarded as a substance analogous to surface pheromones, with specificity range slightly above that of colony or even species odour, in space and time. Further specification of such 'trophic phermomones' possibly makes the symbiotic association between phylogenetically and behaviourally related species[4] possible. While territoriality may be important in the survival of a colony, intercolonial trophallaxis seems vital for the survival of species population as a whole. In such species, the use of radiotracers in demarcating the boundaries of their colonies should be cautioned.

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Comparison of the biological effects of anemia inducing and polycythemia inducing Friend virus complex[1]

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Summary. The comparison of the biological effects of FV^P and FV^A showed that leukemogenesis appears to be delayed in FV^A infected mice as compared to FV^P infected animals after injection of comparable quantities of virus as measured in spleen focus forming units. In addition, no CFU-EI, characteristic for FV^P induced leukemia, were found in leukemic spleen or bone marrow of FV^A infected mice. Since it was possible to distinguish both viruses by their different host ranges, which are helper virus determined, it is suggested that the observed differences, especially the lack of CFU-EI in FV^A infected mice, might be due to differences in the helper virus component of the FV complex.

Abbreviations: CFU-E, colony forming unit-erythroid; CFU-EI, colony forming unit-erythroid (erythropoietin independent); Ep, erythropoietin; FFU, focus forming unit(s); FV, Friend virus complex; FV^A, anemia inducing Friend virus complex; FV^P, polycythemia inducing Friend virus complex; LLV-F, Friend helper virus; SFFV, spleen focus forming virus.

The Friend virus complex (FV) consists of a replication defective transforming component, the spleen focus forming virus (SFFV) and a helper virus, which is a murine lymphatic leukemia virus (LLV-F)[4-9]. 2 basic strains of FV are known, the original isolate of C. Friend[10], which causes anemia (FV^A) and the polycythemia inducing Friend virus (FV^P), which was isolated by Mirand et al.[6,7]. This virus causes an Ep-independent polycythemia in mice[7].

Since the development of polycythemia after infection with FV^P is Ep-independent, that is, non-suppressible by hypertransfusion, the suggestion was made 'that the virus can induce the commitment of erythroid precursor cells independently of erythropoietin'[11].

More recently this was shown to be the case[12,13], since Ep-independent erythroid precursors (CFU-EI) were found in FV^P induced-leukemia. Therefore we asked the question whether CFU-EI are also present in FV^A-induced leukemia, the anemia being a secondary phenomenon only. Our results show that CFU-EI are absent during FV^A-induced leukemogenesis. Therefore the generation of CFU-EI is unique for FV^P. FV^A on the other hand seems to be more closely related to Rauscher virus with respect to its biological properties[14,15].

Material and methods. Virus. The N tropic variant of the polycythemia inducing strain of FV (FV^P) (obtained by courtesy of Dr R.A. Steeves) and the original isolate of C. Friend (obtained by courtesy of Dr W. Ostertag) were grown in DBA/2 mice. FV^P was a spleen focus forming N tropic virus and FV^A a spleen focus forming NB tropic virus as determined in the spleen focus test[4] using DBA/2 and BALB/c mice for the comparison of the host range of both viruses.

Mice. DBA/2 and BALB/c mice were either from Gl. Bomholtgaard, Ry, Denmark or Zentralinstitut für Versuchstierzucht, Hannover, Germany. For the purpose of virus infection DBA/2 mice received an i.v. injection of 1–2×10^4 FFU of FV^P or about 1×10^4 FFU of FV^A.

CFU-E assay. The method described by Iscove[16] was used. 0.8% methylcellulose, 30% fetal calf serum, EP Step III (Connaught Laboratories), 0.2 units/ml, αthioglycerol at an end concentration of 10^{-4} M and bone marrow or spleen cells in the α-modification of Eagle's medium were mixed. 4 parallel petri dishes with 1 ml of medium and 3×10^5 cells were set up and incubated for 48 h at 37 °C in a humidified atmosphere containing 5% CO_2. CFU-EI were quantified in

the same culture system, except that Ep was omitted. Erythroid colonies containing more than 8 small cells were scored without staining at 80-fold magnification.

Results and discussion. 2 groups of DBA/2 mice either infected with FV^P or FV^A were studied and spleen weight, spleen and femur cell numbers, hematocrit, reticulocytes and CFU-E were determined on different days after infection. During leukemogenesis an increase in spleen weight, spleen cell numbers and reticulocytes was observed. However, in case of FV^A infected animals the increase was delayed and the reticulocytosis less pronounced (table 1). On the other hand a moderate increase in femur cell numbers was apparent in the FV^A infected group, while femur cell numbers after FV^P-infection remained at control levels (table 1). At day 8 after infection the hematocrit was already elevated in FV^P infected mice and slightly reduced in the FV^A infected group and the hematocrit values continued to diverge at later time points after infection (table 1).

CFU-E numbers were in general reduced in bone marrow of both groups during leukemogenesis, as compared to controls while a tremendous increase of CFU-E was observed in the spleen of infected animals (table 2). An Ep-independent population of CFU-E (CFU-EI) appeared in spleen and bone marrow of FV^P infected mice already at day 4 after infection while FV^A infected animals lacked this leukemic cell population. At day 8 after infection the majority of CFU-E in FV^P infected mice was Ep-independent, as demonstrated by the observation that cultures with and without Ep contained about the same numbers of CFU-E (table 2).

Our results suggest that leukemogenesis might be delayed in FV^A infected animals as compared to FV^P infected mice receiving comparable quantities of virus (as determined in the spleen focus test).

However, the titer of SFFV allows no conclusion about the relative proportions of Friend helper virus in both virus preparations. In addition qualitative differences between the two helper viruses exist, as demonstrated by their different host range (N and NB respectively) which is determined by the helper virus[5].

3 basic isolates of spleen focus forming viruses exist, Rauscher virus, FV^A and FV^P, 2 of which are very close with respect to their biological properties (Rauscher virus and FV^A)[14] while FV^P infection induces polycythemia, Ep-independent erythropoiesis and the generation of CFU-EI[7,12,13]. Since all these viruses share the SFFV component, it is tempting to assume that the specificity of the biological differences is located in the helper virus component of these viral complexes. Our observation concerning the host range differences of FV^A and FV^P lends support to this hypothesis. This assumption is further supported by work with SFFV transformed nonproducer Friend cell lines[9]. Rescue of the SFFV component by different helper viruses demonstrated that the capacity for the generation of Ep-independent erythropoiesis is probably located in the helper component of the FV-complex (R. Steeves, personal communication). However, the question, why the helper virus of FV^P alone does not cause polycythemia and an Ep-independent erythropoiesis[6], remains unsettled. C57Bl adapted FV^P, on the other hand, was capable of inducing polycythemia in C57Bl mice without extensive SFFV replication[8].

Table 1. Spleen weight, hematocrit, spleen and femur cell numbers and reticulocytes after infection of DBA/2 mice with FV^P or FV^A

Days after infection		Spleen weight* (mg)	Spleen cell* No. ($\times 10^8$)	Hematocrit* (%)	Reticulocytes* (‰)	Femur cell* No. ($\times 10^6$)
	Control	90.4± 17.0	1.17	49.9±1.3	32±21	10.9
4	FV^P	208.1± 13.9	2.2	47.3±2.1	14± 6	9.2
	FV^A	164.7± 15.1	1.6	48.5±1.2	47±30	8.8
8	FV^P	1415.5±232.8	14.9	54.8±0.5	173±48	7.6
	FV^A	424.7±119.1	4.7	46.0±1.0	25± 9	9.0
11	FV^P	1841.4±305.8	14.7	66.7±7.6	324±36	10.6
	FV^A	733.3±163.6	9.4	44.3±2.9	52±17	12.5
15	FV^P	1301.7±280.6	8.6	70.8±3.7	396±40	10.2
	FV^A	1428.0±392.8	17.4	39.3±1.0	142± 9	14.2
20	FV^P	1260.5	12.9	73.0		10.8
	FV^A	1021.4±229.9	6.9	41.3±1.3	106±15	16.1

* Values are averages of 4 mice (± SE). (Uninfected controls: 2 mice at each experimental point.)

Table 2. CFU-E and CFU-EI after infection of DBA/2 mice with FV^P or FV^A

Days after infection		Per femur* Total CFU-E	CFU-EI	Per spleen* Total CFU-E	CFU-EI
4	Control	17513±1118	0	64350± 4505	0
	FV^P	3412± 530	1229± 123	666600± 26664	490600± 39248
	FV^A	3696± 227	0	176000± 5288	0
8	Control	7722± 300	0	31590± 10109	0
	FV^P	6992± 480	9424±1216	18103500±1086210	14850523±1485052
	FV^A	1523± 171	0	6354400	0
11	Control	35484±1897	0	88920± 8892	0
	FV^P	10480± 608	16091±1001	7703300± 308132	6600300± 330015
	FV^A	6996±3018	0	15218600± 760930	0
15	Control	-	-	38610± 1158	0
	FV^P	-	-	1831800± 201498	1599600± 159960
	FV^A	-	-	34974000±2448180	0
20	Control	35252±1763	0	26910± 2691	0
	FV^P	-	-	- -	-
	FV^A	9660± 966	0	11523000± 345690	0

* Values are averages of 4 mice (± SE). (Uninfected controls: 2 mice at each experimental point.)

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Etude ultrastructurale de la transformation du corps élémentaire en corps initial chez les *Rickettsiella*

An ultrastructural study of the transformation of elementary bodies of *Rickettsiella* into initial bodies

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Summary. An intermediate stage between the elementary and the initial bodies of the *Rickettsiella* genus is defined as the beginning of an intracellular cycle. It is characterized by several structural changes in the dense elementary body: the cytoplasm becomes less electron-dense; thus, the nucleoid and the ribosomes are visible. The inner layer of the cell-wall becomes progressively clearer and the trilamellar structure of the inner and outer membranes appears distinctly. 'Pre-initial body' is proposed as name of this stage of development.

Parmi les procaryotes intracellulaires à parasitisme obligatoire, l'ordre des Chlamydiales se caractérise par la présence d'un cycle de développement intravacuolaire[2]. Les mêmes particularités se rencontrent chez le genre *Rickettsiella* (Philip 1956) qui regroupe plusieurs agents pathogènes d'invertébrés appartenant à des classes et des ordres divers (insectes, crustacés, arachnides, myriapodes). Chez ces procaryotes un cycle intravacuolaire de développement marqué par la succession de différentes formes a été décrit[3] et au cours d'une étude comparative, Devauchelle et al.[4] ont mis en évidence l'analogie récemment confirmée[5] du cycle des *Rickettsiella* avec celui des *Chlamydia* ainsi que celle des diverses formes qui se succèdent (forme dense: corps élémentaires, forme de multiplication: corps initial ou corps réticulé, forme intermédiaire: corps intermédiaire).

Chez les Chlamydiales en général, de même que chez les *Ricksettsiella*, le début d'un nouveau cycle infectieux qui se caractérise par la transformation du corps élémentaire en corps initial n'est pas connu avec précision. Ce fait est probablement dû à la très faible durée de cette phase et au petit nombre de cellules contaminées en début d'infection.

Dans cette note, nous décrivons cette phase du passage du corps élémentaire en corps initial marquée en particulier par des modifications du système membranaire. Pour ce travail nous avons choisi d'étudier au microscope électronique *Rickettsiella armadillidii*[6] au cours de l'infection du tissu interstitiel de son hôte, l'isopode *Armadillidium vulgare* Latreille.

Matériel et méthodes. L'isopode *Armadillidium vulgare* élevé à 20 °C et à 65% d'humidité relative est nourri de feuilles de marronniers en décomposition ainsi que de rondelles de carottes et de laitues[7].

Les infections sont faites au moyen d'un microinoculateur en injectant dans la cavité générale des isopodes une suspension riche en corps élémentaires de rickettsies. Pour obtenir ces dernières, les tissus d'animaux préalablement infectés sont prélevés aseptiquement et dispersés dans une solution physiologique. Le tissu interstitiel des oniscoïdes infectés est prélevé 3, 5, 7, 9, 11 et 18 jours après la contamination et fixé dans le glutaraldéhyde à 2% en tampon cacodylate 0,1 M (pH 7,4) additionné de 0,015% de chlorure de calcium. Cette dernière substance, dont l'adjonction est préconisée pour la fixation des procaryotes[8] est favorable en particulier à la préservation des lipides membranaires[9]. La post-fixation est effectuée dans du tetroxyde d'osmium à 1% dans le même tampon. L'inclusion est faite en épon. Les coupes ultrafines sont contrastées par l'acétate d'uranyle et le citrate de plomb.

Résultats. Le corps initial (figure 3) mesure environ 1 µm de long et plus de 300 nm de large. Cette cellule rickettsienne est limitée par une enveloppe dont la structure est composée de 7 feuillets (4 denses et 3 clairs) nommés f1 à f7 de l'extérieur vers l'intérieur[5]. Ces feuillets sont, dans leur ensemble, aisés à distinguer chez les corps initiaux. La paroi est constituée par une membrane externe trilamellaire (feuillets f1 à f3) suivie d'une couche interne formée par le feuillet f4 dont la densité aux électrons est très faible et dont l'épaisseur est assez irrégulière. La membrane plasmique est trilamellaire (feuillets f5 à f7).

Le corps élémentaire infectieux est ovoïde et de petite taille (figure 1). Il mesure en moyenne 475 nm de long et 175 nm de diamètre. Il se caractérise par son contenu très dense aux électrons et par son système membranaire condensé dont l'épaisseur totale est de 22 nm et qui est composé par l'alternance de 3 feuillets denses opaques d'inégale épaisseur, et de 2 feuillets clairs. Le feuillet dense médian, le plus épais, correspond en fait à la juxtaposition des 3 feuillets f3, f4 et f5. En effet, le feuillet f4 présente ici une forte densité aux électrons, liée en particulier à une accumulation de polyosides qui se produit lors de la transformation du corps initial en corps élémentaire, en fin de développe-