Table 2. The absence of cross reactivity between the Potomac horse fever agent (PHFA) and specific antisera to 12 rickettsiae as determined by the test. Except where indicated, the antisera were produced in rabbits. N, no reaction at a serum dilution of 1:10.

Antiserum to	Homologous titer*	IFA test with PHFA antigen
Rickettsia tsutsugamushi (Kato)	1:300,000	N
R. tsutsugamushi (Karp)	1:300,000	N
R. tsutsugamushi (Gilliam)	1:300,000	N
R. prowazeki	1:20,000	N
R. typhi	1:20,000	N
R. canada	1:30,000	N
R. conorii	1:30,000	N
R. akari	1:30,000	N
R. rickettsii†	1:8,192‡	N
Rochalimaea quintana	1:300,000	N
Rochalimaea vinsonii	1:300,000	N
Coxiella burnetti†	1:512†	N

*Except when indicated, the titer was determined by an enzyme immunoassay. †Antiserum obtained from naturally infected humans. ‡Titer determined by microagglutination.

To obtain conclusive evidence that the newly isolated organism is the causative agent of Potomac horse fever, we inoculated a susceptible pony with culture-derived organisms obtained from the third consecutive subpassage in primary canine monocyte cultures (*13*). The animal developed a fever (102° to 104.2°F) on day 7 after inoculation and by day 12 became depressed and anorexic. At this time diarrhea and slight edema on the distal portion of the hind legs were present. By using the methods described above we reisolated the organism from this animal in cultures initiated on days 10 and 12 after inoculation. The animal was killed on day 16 after inoculation and an autopsy revealed pathological manifestations consistent with Potomac horse fever (*14*). When a similar number of cells from control, noninfected canine monocyte cultures from the same donor dog were inoculated into another susceptible pony, no clinical, hematologic, or serologic signs of the disease were evident.

The name Potomac horse fever was chosen to describe the location of the earliest cases of the disease. In view of the occurrence of the disease in other parts of the United States and possibly in Europe, we suggest that the name equine monocytic ehrlichiosis would be more descriptive. This name would be clearly distinguishable from equine ehrlichiosis caused by *E. equi* (*6, 15*).

The discovery of the causative agent of equine monocytic ehrlichiosis and culture of the organism in vitro will facilitate the development of effective control measures including chemotherapeutics and, eventually, immunoprophylactics. It may now be possible to use serological tests to detect subclinical and overt forms of the disease and to determine its true geographic distribution. Since the causative organism is antigenically related to *E. sennetsu*, a human pathogen that has not, thus far, been demonstrated to occur in the western world (*6*), it may be necessary to examine the public health importance of the new agent.

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4. The agent described here has been maintained by serial blood transfusions from acutely infected to susceptible ponies at National Veterinary Services Laboratory (NVSL), Ames, Iowa.
5. Serum samples obtained from horses before and after they were experimentally infected with the Potomac horse fever agent as well as samples from naturally infected animals were examined in our laboratory for the presence of antibodies against *E. equi*, *E. canis*, and *E. sennetsu* by means of the IFA test.
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11. Volumes (1 ml) of supernatant medium from four infected equine monocyte cultures were transferred to primary canine monocyte cultures. The cultures were incubated for 1 hour at 37°C at which time 4 ml of fresh culture medium containing 20 percent normal dog serum was added to each flask.
12. Antisera against 12 rickettsiae were kindly provided by E. Weiss, U.S. Naval Medical Research Institute, and W. Burgdorfer, NIH, Rocky Mountain Laboratory.
13. A suspension of approximately 2×10^4 infected canine monocytes was centrifuged at 400*g*. The supernatant fluid was removed. The cell pellet was resuspended in 4 ml of sterile Hanks balanced salt solution (HBSS-Gibco). The cell suspension was then injected intravenously into a susceptible pony.
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Histological Demonstration of Mosaicism in a Series of Chimeric Rats Produced Between Congenic Strains

Abstract. *Experimental chimeras were produced by aggregating morulae from congenic strains of PVG rats differing in the major histocompatibility complex (RT1). Monoclonal antibodies against variant class I antigens of the two strains were directly conjugated to iodine-125 and applied to tissue sections. Autoradiograms allowed examination of most internal tissues. The proportion of PVG-RT1^a cells in the erythrocyte populations of the chimeras varied from 8 to 70 percent, as determined with fluorescence-activated flow cytometry. Digital analysis of autoradiograms demonstrated that the contribution of PVG-RT1^a cells to the livers of the chimeras ranged from 34 to 86 percent. Patches of cells of each genotype in the liver were geometrically complex, with large variations in size. The thymus, but not the spleen, showed evidence of oligoclonal development. The adrenal cortex revealed a radially striped pattern, suggestive of clonal expansion of stem cells. With this approach it is possible to measure cell distribution in chimeras through direct histological visualization, which may prove useful in the study of rat organogenesis.*

Chimeric (allophenic) animals have been produced experimentally in a number of mammalian species. Such animals are prepared by amalgamating two or more preimplantation embryos in vitro and transferring the chimeric embryos to surrogate mothers for intrauterine development (*1*). The adult tissues of these animals are mosaics composed of the progeny of the two or more cell lineages present in the original embryos. Analysis of the mosaic tissues has been used to

answer certain developmental questions (*2–4*). This has been a particularly useful experimental model in the case of chimeric mice, which have been constructed in many laboratories.

Mammalian chimeras have been produced in other species, including sheep (*5*), rabbits, (*6*), and rats (*7*), and some interspecific chimeras have been achieved (*8*). In general, it has proven more difficult to produce chimeras in species other than mice, and such animals have been more difficult to analyze. This is due to a variety of factors, including difficulty in obtaining and culturing embryos as well as the paucity of markers in the genetically less well defined species. To exploit the marker systems available, most mammalian chimeras have been produced between widely different inbred strains or between outbred individuals. The implications of such genetic inhomogeneity are not known. There is a need for a genetically defined marker system that can be used histologically to identify parental origin in chimeras. Surprisingly, class I antigens of the major histocompatibility complex (MHC), which are well-expressed on the surface of nearly all tissue cells, have only recently been used as histological markers for chimeric mice (*9*), and never, to our knowledge, in other species.

There have been at least four reports of chimeric rats (*7, 10–12*). The largest series was that of Yamamura and Markert (*7*), who described a successful culture medium for the in vitro development of rat preimplantation embryos. We used these conditions to produce a series of allophenic rats between two congenic inbred strains differing only at MHC RT1. We found that the histological distribution of the two parental components can be visualized in several tissues with radioiodinated monoclonal antibody directed against the class I MHC molecule of one parent. A particularly clear-cut distribution of parental components is shown in the liver, adrenal cortex, and thymus. For the thymus preliminary evidence is presented to suggest that the normal thymus is oligoclonal, with independent stem cell colonization of the cortex and medulla, as recently proposed on the basis of data obtained from radiation chimeras (*13*).

Of 474 embryos isolated from 62 donor females, 298, or 62.9 percent, were healthy and at the eight-cell stage. In all, 80 pairs of embryos were aggregated; 59 of these, or 73.8 percent successfully formed giant morulae or blastocysts after 18 to 24 hours in culture. A total of 50 of these pairs were transferred to six recipient females. Thirteen pups were born in three litters: four of these died within 24 hours and one within 7 days. None of these animals had demonstrable mosaicism of their tissues. The remaining eight animals were analyzed as described below.

Table 1. Analysis by FACS of RBC populations from viable offspring. The antibodies used are defined in the legend to Fig. 1. RBC's were washed in phosphate-buffered saline (PBS) and incubated on ice with R3/13 or YR5/12 diluted in PBS and fetal calf serum. Control samples were incubated with the diluent alone. Cells were then washed and incubated on ice with FITC-labeled mouse monoclonal antibody to rat IgG2b (*19*). After being washed, cells were fixed in 1 percent buffered formaldehyde and analyzed in the FACS (Ortho).

Animal	PVG-*RT1*^{a} cells (%)
Parent PVG-*RT1*^{a}	99.0
Parent PVG-*RT1*^{c}	4.2
0903	7.5
0904	2.0
0906	70.4*
0907	2.2
0909	59.7*
0910	0.9
0911	36.6*
0912	7.5*
Mean system background	1.8

*Unequivocal chimera (two clearly distinguishable RBC populations).

The results of fluorescence-activated cell sorter (FACS) analysis of red blood cells (RBC's) from the eight viable offspring and parental controls are summarized in Table 1. The background distribution due to fluorescein isothiocyanate (FITC)–labeled antibody to rat immunoglobulin G2b (IgG2b) was measured for each red cell population and the mean was used for comparison. There was unequivocal evidence of RBC chimerism in four of the eight animals. Examples of the distribution of fluorescence in these RBC populations are shown in Fig. 1.

All tissues of the parental animals, with the exception of the brain, showed uniform reaction of PVG-*RT1*^{a}–derived tissues autoradiographically with iodinated monoclonal antibody R3/13 or R2/15S (see legend to Fig. 1). PVG-*RT1*^{c}–derived tissues showed uniform absence of reaction with these antibodies (*14*).

The livers of all of the chimeric animals were sampled for histological analysis, and the tissues described below were obtained from animal 0911. Table 2 shows the results of digital analysis of sections of the livers, demonstrating the range of proportions of parental cell types, number of patches per unit area, and average patch size.

There was clear evidence for patchy chimerism in the thymus. In general there was more intense labeling with R2/15S in medullary than in cortical areas, as expected from the known differential expression of class I MHC molecules

Table 2. Digital analysis of histological sections of liver from the four chimeric offspring. Unstained autoradiograms of the frozen tissue sections were photographed on positive transparency film and projected onto a digitizing tablet through a front-surfaced mirror. The tablet was interfaced with a 16-bit microcomputer and contiguous areas of PVG-*RT1*^{a}– or PVG-*RT1*^{c}–derived cells were traced with the tablet cursor. Areas, area ratios, and standard deviations were computed from the *x,y* coordinates generated by the digitizing tablet with a program developed by L. Berkwits, Northwestern University Medical School.

Section	PVG-*RT1*^{a} cells (%)	Number of patches per unit area	Average patch area (mm^2, mean ± standard error)	*N*
	Animal 0906			
11	88.65	53.36	0.0022 ± 0.0004	58
12	86.03	32.74	0.0042 ± 0.0008	42
13	82.80	34.00	0.0045 ± 0.0008	51
Mean ± standard error	85.83 ± 1.69	40.03 ± 6.67		
	Animal 0909			
11	53.37	18.17	0.0197 ± 0.0045	20
12	42.80	22.71	0.0156 ± 0.0035	32
13	61.57	8.68	0.0288 ± 0.0071	8
Mean ± standard error	52.58 ± 5.43	16.52 ± 4.1		
	Animal 0911			
11	74.78	28.48	0.0087 ± 0.0016	33
12	74.18	30.63	0.0090 ± 0.0032	41
13	76.09	32.26	0.0082 ± 0.0029	39
Mean ± standard error	75.02 ± 0.56	30.46 ± 1.09		
	Animal 0912			
11	37.35	29.03	0.0118 ± 0.0033	43
12	35.84	30.81	0.0114 ± 0.0036	48
13	29.45	30.36	0.0090 ± 0.0023	45
Mean ± standard error	34.21 ± 2.42	30.07 ± 0.53		

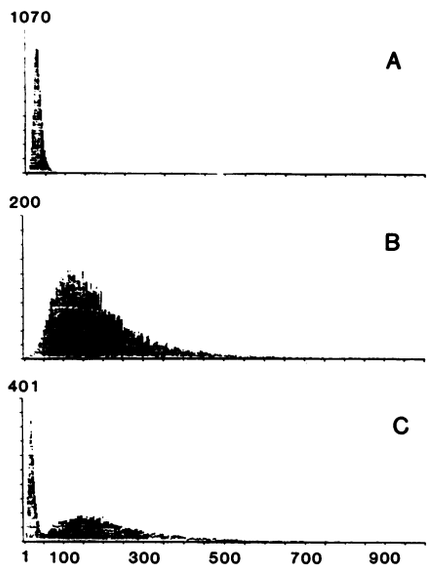
among lymphocytes from these regions. In the cortical regions the distribution of labeling was consistent with the independent oligoclonal origin of lymphocytes in each lobule. The upper lobule appeared to be uniformly of PVG-*RT1*^{a} origin, while the lower lobule contained several discrete patches of the PVG-*RT1*^{c} type (Fig. 2A). The medullas of both lobules were predominately of PVG-*RT1*^{a} origin. The appearance of the lower lobule was consistent with invasion of the medulla across a broad front by cells migrating from the cortex, since the cortical patches of PVG-*RT1*^{c} origin appeared to extend into adjacent medullary regions. There is histological evidence from this chimera for independent colonization of the medulla by stem cells, as recently reported by Ezine *et al.* (*13*) in mouse radiation chimeras.

There was a random pattern of patchy chimerism in the major sublingual and submaxillary salivary glands (Fig. 2B). The spleen demonstrated chimerism of both the white and red pulp (Fig. 2C). The class I determinant density of the white pulp, consisting of lymphoid cells, was higher than that of cells of the red pulp, consisting mainly of erythrocytes, phagocytes, vessels, and other structural elements. Chimerism in the periarteriolar sheaths was seen as fine stippling, as expected from the principal traffic areas for recirculating lymphocytes. There was no evidence for an oligoclonal origin of germinal center cells. The red pulp also showed a fine admixture of both cell types.

The liver sections demonstrated a consistent pattern of chimerism, with well-formed patches whose shape, size, and distribution were commensurate with the proportions of the two cell types in the organ (Fig. 2D). There was no evidence of zonal distribution of the patches in the organ. Digital quantitation was performed with unstained autoradiograms of the liver from all four chimeras (Table 2). The reported relation between the proportion of parental cell types and the number of clones per patch in chimeric tissue (*15*) was seen in this series of chimeric rats.

The adrenal gland showed a peculiar pattern of chimerism (Fig. 2E). The medulla did not react with antibody to RT1A^a antigen, consistent with the neuroectodermal origin of this tissue. The adrenal cortex, on the other hand, showed a striped pattern of reaction, with alternating regions of PVG-*RT1*^{a} and PVG-*RT1*^{c} cells. This pattern is most consistent with a random assortment of primordial cells on the surface of the medulla and subsequent clonal outward proliferation of cells to form the adult tissue. A similar pattern and explanation have been reported for coat color in chimeric mice (*2*). The stripes extended through all three layers of the adrenal cortex (zona reticularis, zona fasciculata, and zona glomerulosa) and were parallel to the cords of secretory cells in the zona fasiculata.

The kidney showed a pattern of random patchiness suggestive of embryological intermixing of cells similar to that



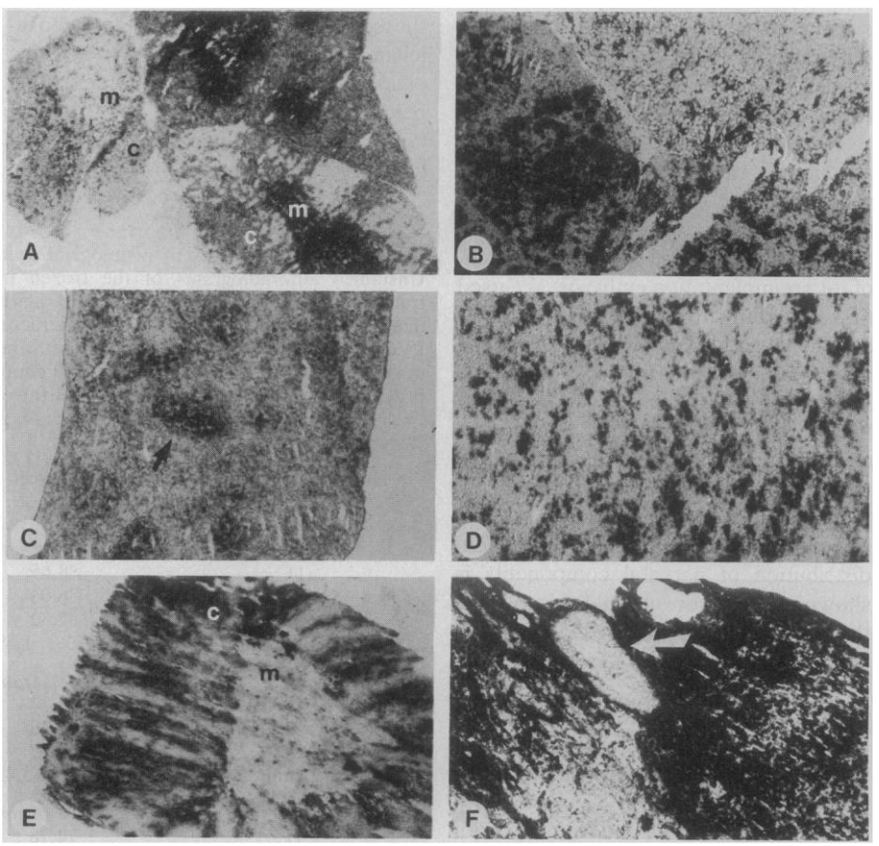


Fig. 1 (left). Histograms derived from FACS analysis of RBC populations from chimeric and parental animals. (A) Red blood cells from a PVG-*RT1*^{c} animal. All the cells have little or no fluorescence. (B) Red blood cells from a PVG-*RT1*^{a} animal. The cells show a normal distribution of fluorescence. (C) Red blood cells from chimera 0906. The system background has been subtracted. The IgG antibodies directed against the class I molecule of the *RT1*^{a} haplotype have been described previously (*18*). R2/15S is an IgG2a while YR/100 and R3/13 are IgG2b's. YR5/12 is an IgG2b monoclonal antibody directed against the class I molecule of the *RT1*^{c} haplotype. Purified R2/15S antibody was iodinated with chloramine-T under conditions that minimize oxidative damage to the molecule. Fig. 2 (right). Photomicrographs of unstained autoradiograms, made after incubation of frozen tissue sections with iodinated R2/15S antibody. (A) Thymus, showing both cortical (c) and medullary (m) regions. (B) Major sublingual and submaxillary glands. (C) Spleen. The white pulp (arrow) stains more intensely than the red pulp, but all areas show fine variegation of cells of the two parental types. (D) Liver. Discrete patches of PVG-*RT1*^{a} cells are evenly dispersed through PVG-*RT1*^{c} tissue. Autoradiograms of liver from the other chimeras demonstrate more complicated patch construction. (E) Adrenal gland. The medulla is unreactive, consistent with its neuroectodermal origin. The striped appearance of the cortex suggests the pattern of organogenesis of this organ. (F) Ovary. Discrete patches of the two parental cell types are evident throughout the stroma of this organ. Graafian follicles (arrow) are very weakly labeled. Magnification: ×16.8 (A to E) and ×26.9 (F).

of the other solid organs, such as the liver. There did not appear to be any specific localization of either genotype lineage in this organ.

A random pattern of patch distribution observed in the lungs was unrelated to structural elements in this organ. The patches were uniform in size and shape and were similar to those in the liver.

The cortex and medulla of the ovary had a random patchy distribution of cells of the two genotypes. The graafian follicles were unstained, consistent with the absence of MHC antigens in germ cells (Fig. 2F). Chimera 0911 had mature follicles and numerous primary and secondary follicles. Its oviduct contained an ovulated germ cell demonstrating germinal vesicle breakdown and corona radiata. Of the eight live births, five were female and three were male. All four of the unequivocal chimeras were female. Two of the three surviving females have mated with male Holtzman rats and have given birth to a total of 18 pups in six litters.

There are three systems that allow histological examination of chimeric tissues other than the liver (*8, 9, 16*). Interspecific chimeras suffer the disadvantage of having disparate genetic backgrounds with possible effects on normal cell assortment in the organogenetic phase of development. This does not affect the function of at least the vital organs of such interspecific chimeric animals in any determinate manner. The use of congenic strains that vary genetically only at or near the marker locus obviates this limitation. Morphometric analyses of specific organs in chimeric mice have been limited to the liver (*17*). Our results in the rat liver tend to support the conclusions of these studies concerning average patch size, patch distribution, and the relation between the proportion of the two genotypes present and the patch size.

The combination of MHC antigens in congenic animals marked histologically with monoclonal antibodies provides a useful addition to the already widespread developmental applications of mammalian chimeras and may provide new insights into the organogenesis of some viscera. This may be particularly true of the thymus, for which evidence of oligoclonal development, which has important implications for the tempo of T-cell repertoire formation and the induction of self-tolerance, is currently derived solely from the radiation chimera model. It is possible that accessibility of the thymus to immigrating cells in that situation is atypical, and it is important to evaluate this possibility in normal animals. The system we have described allows histological examination and study of organogenesis of mosaic rat tissues.

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Microorganism Mediated Reproductive Isolation in Flour Beetles (Genus *Tribolium*)

Abstract. *Reproductive isolation is induced by microorganisms in diverse geographic strains of the flour beetle* Tribolium confusum *(Coleoptera:Tenebrionidae). The incompatibility between populations is due to nongenetic cytoplasmically inherited factors. Males of infected strains produce no progeny when crossed with females of noninfected strains; however, they produce "normal" numbers of progeny when crossed with infected females. Males from noninfected strains show no reproductive isolation. Infected strains of* T. confusum *can be cured when tetracycline or other antibiotics are added to the flour medium. "Cured" strains become partially reproductively isolated from all noncured strains including the source strain.*

Incompatibility between populations due to nongenetic factors is of interest because of its possible role in the process of speciation (*1–3*) and because of its potential application to the control of pests (*2*). In the mosquito *Culex pipiens*, cytoplasmic incompatibility is mediated by the prokaryotic microorganism *Wolbachia pipientis* (*1*). Males not containing *Wolbachia* can mate with any female, whereas those containing *Wolbachia* are reproductively isolated from females not containing the microorganism. The antibiotics tetracycline, erythromycin, and spectinomycin inhibit prokaryotic and mitochondrial protein synthesis by binding to the ribosome (*4*); thus *Wolbachia* infected mosquito larvae raised in water containing tetracycline are cured of the microorganism.

In 1961, Stanley (*5*) reported that females of the McGill black *Tribolium confusum* (flour beetle) strain were reproductively incompatible with two other strains, although males of this strain were not. Moreover, McGill black females were also incompatible with the hybrid males produced by crossing McGill black males with females of other strains. Cawthon and Mertz (*6*) discovered a similar phenomenon in the bI *T. confusum* strain. He showed further that the infertility exhibited a cytoplasmic pattern of inheritance: bI females were infertile with F_1, F_2, and F_3 backcross hybrid males derived from mating bI males with Chicago strain females. [The bI strain was derived from the Chicago strain by inbreeding (*7, 8*).]

In 1979, the mating compatibilities of 12 strains of *T. confusum* at the University of Chicago were surveyed. Six of the strains, including bI and Chicago, had been in the laboratory for more than 20 years. The other six strains had been recently collected from stored grain