Charles C Thomas, Springfield, 1962.
2. Schueler, F., *J. Pharmacol. Exp. Therap.*, 1955, v115, 127.
3. MacIntosh, F. C., Birks, R. I., Sastry, P. B., *Nature*, 1956, v178, 1181.
4. ———, *Neurology*, 1958, v8, (Supplement), 90.
5. Gardiner, J., *J. Physiol.*, 1957, v138, 13P.
6. MacIntosh, F. C., *Fed. Proc.*, 1961, v20, 562.
7. Reitzel, N. L., Long, J. P., *Arch. Intern. Pharmacodynamie*, 1959, v119, 20.

Received January 8, 1964. P.S.E.B.M., 1964, v115.

Response of Normal and Immune Histiocytes of Various Animal Species to Infection by *Brucella melitensis.** (29063)

SANFORD S. ELBERG, PATRICIA MASCARENHAS AND JACOB FONG
Department of Bacteriology, University of California, Berkeley

One has attempted to observe the relation which exists between peritoneal histiocytes and *Brucella melitensis* after uptake of the latter by normal and immune cells *in vitro*. To this end maintenance and growth of the histiocytes are essential for relatively long periods in order to be certain of the vitality of the host cell, although the end-point of the experiment is available within 3-4 days of incubation. Indeed the major outlines of the response of histiocytes to the cytotoxicity of the brucellae are manifest within the first 24-hour period of incubation, after which further degeneration or continued survival followed by multiplication is principally a function of the relative virulence of the brucellae.

The data will show that peritoneal histiocytes from rabbit, monkey and guinea pig do not differ in their cytotoxic response. This response is non-specific in terms of the species of brucella being studied for immunization and challenge infection.

Methods. Histiocytes were harvested and treated as previously described by Elberg *et al*(1). The cells were exposed to *B. melitensis*, strain Rev I, which had been grown on Albimi agar and washed 3 times in modified Tyrode solution.† A ratio of 10 bacteria: 1 monocyte was used for parasitization. The cells were then cultivated in either Mackaness or Sykes-Moore microchambers. Normal cells were cultivated in Tyrode + 40% normal rabbit serum; immune cells were grown in 40% immune serum in Tyrode solution. Samples were taken at 3, 24, 72, 120, and 168 hours.

Results. Maintenance was well demonstrated in the chamber cultures. The customary degeneration after parasitization of histiocytes, derived from normal animals, and due to the cytotoxic action of the brucellae, is seen in the data of Table I which presents a sample from many animals and chambers giving identical results.

A comparison of Hartley strain of guinea pig and *Cynomolgus phillipinensis* histiocytes

TABLE I. Survival and Multiplication of Rabbit Peritoneal Histiocytes Following Parasitization by *B. melitensis*, Strain Rev I.*

	0 hr	24 hr	48 hr	96 hr
Non-parasitized (a)	559	561	592	896
normal cells (b)	637	639	675	920
Parasitized (a)	631	461	458	503
normal cells (b)	833	615	601	670
Non-parasitized (a)	396	394	400	700
immune cells (b)	436		443	658
Parasitized (a)	452	450	450	
immune cells (b)	489	493	501	891

* Attenuated vaccine strain.

* Supported by grants from Nat. Inst. Health and Nat. Science Foundation.

† Modified Tyrode Solution: *Solution A:* NaCl, 7.7 g; KCl, .2 g; $MgSO_4$ (anhydrous) .1 g; Dextrose, 4.0 g; NaH_2PO_4, .05 g; Water, 750 ml. *Solution B:* $NaHCO_3$, .5 g; Water, 25 ml. Double-distilled water is used. The solutions are autoclaved separately and combined when *cool*. If an indicator is desired, .1 ml of phenol red (La Motte Solution, 1%) is added to Solution A before autoclaving.

TABLE II. Response of Guinea Pig and Monkey Monocyte Cultures to the Rev I Strain of *B. melitensis*.

	0 hr	24 hr	% deg	48 hr	% deg	96 hr	% deg
(a) Guinea pig*							
Normal cells	509†	502	1	509	0	508	0
	465	463	0	463	0	468	0
	478	474	1	474	0	476	0
" " with brucella	969	679	30	670	0	671	0
	635	432	32	431	0	440	0
	659	476	28	476	0	480	0
Immune cells	269	269	0	269	0	278	0
	269	268	0	269	0	273	0
" " with brucella	500	498	0	498	0	506	0
	241	243	0	244	0	243	0
(b) Monkeys*							
Normal cells	493	494	0	493	0		
	450	458	0	458	0		
	428	430	0	429	0		
" " with brucella	562	372	34	374	0		
	555	358	25	360	0		
	413	293	29	294	0		
Immune cells	436	436	0	450	0		
	485	485	0	489	0		
	386	385	0	386	0		
" " with brucella	500	508	0	507	0		
	476	473	0	475	0		
	497	498	0	498	0		

* Sample of 3 separate experiments.
† Data comprise counts from 3 separate chambers per animal.

for their responses to parasitization by *B. melitensis* was carried out. Histiocytes from normal animals and from animals immunized 3 months earlier with 1 billion cells of the Rev I strain of *B. melitensis*(2) were employed. Each guinea pig received 7 ml of Klearol intraperitoneally. The composition of the medium was 50% serum + 50% Tyrode solution rather than 40%-60%, respectively, as in the experiments on rabbit cells.

The monocytes were harvested, trypsinized, and parasitized with 10:1 ratio of bacteria to cells. This results in about 15-25% of histiocytes becoming parasitized, the optimal ratio for experiments of this type.

Twenty ml of oil were injected intraperitoneally per monkey.

The medium was iced and pH 7.0 maintained while the cells were in suspension before incorporation in micro-chambers.

No differences could be seen in the behavior of the guinea pig and monkey histiocytes from that shown by rabbits (Table II). Morphology and viability for the 96-hour period was well maintained, and the ability of cells from immunized animals to resist initial degeneration which follows uptake of Rev I cells by normal monocytes was characteristic.

The immunity of cells from animals immunized against *B. melitensis* was equally effective against parasitization by *B. suis*, and since the data are typical of those in Tables I and II, they are not presented in detail.

The capacity of histiocytes from specifically-immunized animals to sustain the presence of intracellular brucellae for several days without themselves undergoing degeneration suggests that even in *in vitro* cell populations the immune cells reflect the *in vivo* infection process. In fact, it is not difficult, under the conditions described in this study, to maintain histiocyte cell populations *in vitro* for 3 months or longer, with replacement of medium at 5-day intervals.

The retardation of the multiplication of intracellular brucellae by immune-type cells

TABLE III. Percent of Cells Parasitized by *B. melitensis* Revealed by Modified Machiavello Staining on Preparations from Sykes-Moore Chambers.

Time (hr)	Normal histiocytes	Immune histiocytes
0	(a) 21% (1-2 bact/cell)	25% (1-2 bact/cell)
	(b) 22% (*idem*)	24% (*idem*)
24	(a) 79% (49% 1-5 bact/cell; 30% 5-15 " ")	44% (never more than 10 bact/cell)
	(b) 78% (48% 1-5 bact/cell; 30% 5-15 " ")	52% (*idem*)
48	(a) 90% (all well above 15 bact/cell)	10% (1-5 bact/cell)
	(b) 98% (*idem*)	
72	(a) (b) 95% (many bact/cell)	11% (1-2 bact/cell)
		7% (*idem*)
144	(a) (b) All cells degenerated	100% infected

is nevertheless quite clearly demonstrated, both in terms of numbers of cells parasitized and in terms of numbers of brucellae per cell. This is shown in the data presented in Table III.

Unpublished data showed also that the peritoneal histiocyte induced by peritoneal inflammation is exceeded in its activity by immune alveolar histiocytes.

Fluorescein-labeled specific antiserum was employed along with the Machiavello stain to localize the intracellular brucellae. We could find no advantage between the two procedures in the early weeks of parasitization(3). In the later stages the value of the labeled serum clearly would be to detect antigenic material no longer present as intact cell susceptible to conventional staining.

Summary. Inflammatory-induced peritoneal histiocytes of rabbit, guinea pig and monkey, both normal and immunized, respond to the cytotoxic action of parasitizing brucella within 24 hours. Normal cells undergo about 30% loss whereas immune cells resist completely the cytotoxic action for at least 96 hours and retard intracellular multiplication for at least 144 hours. Cells of rabbits, guinea pigs, and monkeys react alike in these respects. The cellular immunity produced by the Rev I strain of *B. melitensis* was also operative against virulent *B. suis*.

1. Elberg, S., Schneider, P., Fong, J., *J. Exp. Med.*, 1957, v106, 545.

2. Elberg, S., Faunce, W. K., *J. Bact.*, 1957, v73, 211.

3. Janney, G. C., Berman, D. T., *Am. J. Vet. Res.*, 1962, v23, 596.

Received September 3, 1963. P.S.E.B.M., 1964, v115.

Propagation of Western Equine Encephalitis Virus in Mice Following Intramuscular and Intranasal Inoculation.* (29064)

JAMES E. FROESCHLE† (Introduced by Harald N. Johnson)
Viral and Rickettsial Disease Laboratory, California State Department of Public Health, Berkeley

The hypothesis has been proposed that arthropod transmission may not be essential for maintenance of Western equine encephalitis (WEE) virus in the natural wildlife hosts(1). The purpose of this study was to determine the distribution of a non-neuro-adapted strain and a cloned variant of WEE virus in experimentally infected mice and to

* Arthropod-borne Virus Studies, California State Dept. of Public Health, Berkeley. This is a joint project of Rockefeller Foundation and California State Dept. of Public Health.

† This investigation was supported in part by an Epidemiology Training Grant from Nat. Inst. Health, U.S.P.H.S., to School of Public Health, Univ. of California, Berkeley.