

# *Acta Medica Okayama*

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*Volume 13, Issue 2*

1959

*Article 1*

JUNE 1959

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## The cytogenesis of ascitic pha-gocytes

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# The cytogenesis of ascitic pha-gocytes\*

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## Abstract

Judging from our vital observation conducted mainly by tissue culture, it was firmly demonstrated that ascitic phagocytes are not histiocytes but they are the cells closely related to monocytes and that the sites of the genesis are the milky spots of the greater omentum. The milky spots are most possibly the remnants of the mesenchymal hematopoiesis of the embryonic stage.

ActaMed. Okayama. 13, 83-92 (1959)

## THE CYTOGENESIS OF ASCITIC PHAGOCYTES

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*Received for Publication, December 15, 1959*

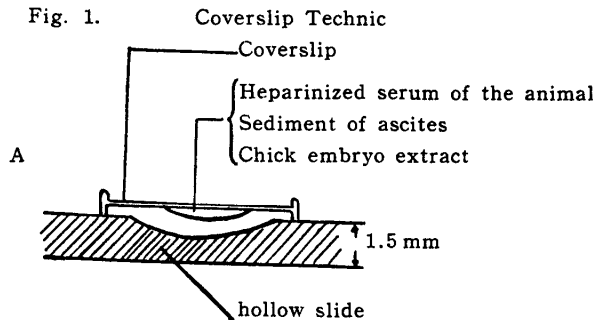
### INTRODUCTION

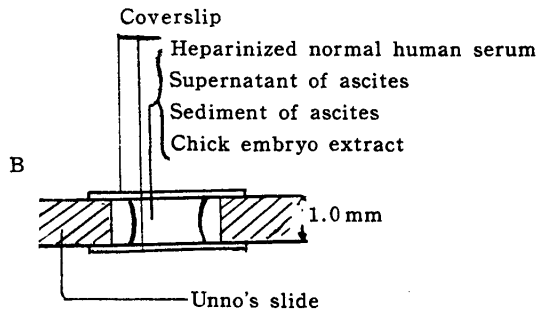
Concerning the cytogenesis of the so-called ascitic phagocytes which occupy 80 per cent of ascites cells, there are two conflicting theories; namely, the one which contends that they belong to histiocytes<sup>1,2,6,7</sup>, and the other which claims them to belong to monocytes<sup>3,5,12,13,14</sup>. The authors have made an attempt to clarify the cytogenesis of these cells mainly with tissue cultures<sup>9,10,11</sup>, of human, dogs, cats, rabbits, albino rats, mice, and chickens. Furthermore, as for the cytogenesis of the milky spots there are two opposing theories as in the case of ascitic phagocytes namely histiocyte theory<sup>1,2,7</sup>, and monocyte theory<sup>3,5,12,13,14</sup>, although as for the source of ascitic phagocytes the greater omentum theory<sup>1,2,3,5,7,12,13,14</sup>, is widely believed.

The authors have studied the greater omentum of the animals mentioned above and the greater omentum and the ascitic cells of rabbits in the various embryonic stages.

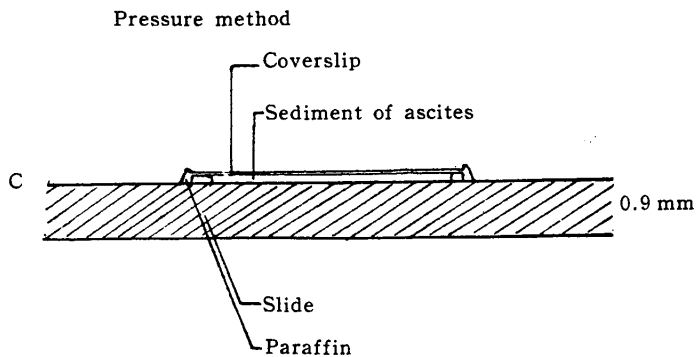
### METHOD

In our tissue culture we used the coverslip method (Fig. 1. A, B) and for substrates, the sera of the subjects, and for nutrients the supernatant





of ascites and the extracts of chick embryo incubated 7 to 9 days, were used. Observations were carried on by a microscope placed in a box kept warm at 37°C; and the movement of cells was scrutinized by taking cinematographs on the 16 mm film. In addition, observations on the stainability to neutral red, Janus green vital stainings as well as phagocytosis of carbon particles were conducted along with fluorescence-microscopic observations by acridine orange, of the same culture. Besides tissue culture the phase-contrast microscopic observations on pressure preparations (Fig. 1. C) were carried on, and also smear preparations and the peroxidase reactions were scrutinized.



#### RESULTS AND COMMENTS

I. *The cell composition of ascites*: As for the cell composition of ascites, as shown (Table 1) phagocytes occupy 80 per cent of the cells in all test subjects, followed by a few of lymphocytes, granulocytes and serosa cells.

II. *The cytogenesis of ascitic phagocytes*: Firstly, the ascitic phagocytes of mice and rats in May-Giemsa preparations have combined cytologic features of histiocytes and monocytes: a dirty cytoplasm and dense

Table 1. Cell number and cell composition of normal ascites of various animals

species	mouse	rat	rabbit	dog	cat	monkey	human
cell No. /mm <sup>3</sup>	74,100	67,221	1,796	3,040			1,161
cell type							
phagocyte	85.4%	80.6%	92.2%	84.0%	86.8%	90.7%	89.0%
{ large	9.3	4.0	8.3	8.0	8.0	5.9	11.1
{ medium	57.8	64.3	69.8	65.0	60.0	70.6	62.2
{ small	18.3	12.2	14.1	11.0	18.8	14.2	15.7
lymphocyte	10.9	5.5	6.8	7.0	12.0	7.4	8.0
serosa cell	0.08	0.02	0.15	—	—	—	0.2
neutrophil	0.2	0.2	—	7.0	12.0	12.0	1.1
pseudoeosinophil	—	—	0.8	—	—	—	—
eosinophil	2.2	7.7	—	—	—	—	1.0
basophil	—	—	—	—	—	—	—
mast cell	1.2	6.0	0.01	—	—	0.7	0.7

chromatin networks belong to the former and a thin nuclear membrane and location of azur granules in the nuclear indentation to the latter. In higher animals, however, in the order of rabbits, cats, dogs (Fig. 2), monkeys, and man, the cytoplasm becomes clearer, and nuclear lobulation tends to be more marked, and unclear networks growing far finer; thus they come to resemble more like monocytes. Even in the case of mice, furthermore, the new cells of ascitic phagocytes appearing after irritation of the peritoneal cavity by typhoid vaccine and lactic acid resemble remarkably to monocytes. (Fig. 3) As for the peroxidase reaction (Table 2), in all animals whose monocytes are positive to this reaction, these ascitic cells are also positive.

Table 2. Percentage of positive peroxidase reaction in normal ascitic phagocyte of various animal

species	mouse	rat	rabbit	dog	cat	monkey	human
total of phagocyte	18.4%	14.0%	0.5%	22.6%	19.0%	23.8%	20.7%
{ large	2.30	1.6	0.2	3.8	1.0	2.8	3.8
{ medium	12.02	11.0	0.3	16.0	14.0	17.8	13.9
{ small	4.07	2.3	0	2.8	4.0	3.2	3.0

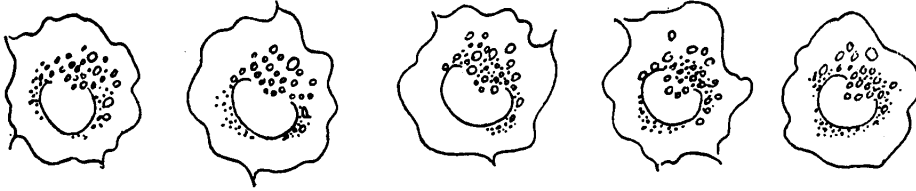
Next, we have observed the pattern of cell movement in tissue culture<sup>4,8</sup>. In our previous bone-marrow tissue culture of man as well as of various animals, we had taken cinematographs of the patterns of various

cell movements, and we classified them into type A to I and pseudopodia into type I to V, running the film at the rate of 1 frame per 1 to 2 seconds. Monocytes present D-type movement, the pattern peculiar to them (Fig. 4). Namely, by protruding the membranous pseudopodia from the entire cell periphery and slowly flagging these, they wander about. In addition, monocytes protrude and retract other longer tentacle-like pseudopodia. This pattern of monocyte movement is about the same in all animals. On the other hand, subcutaneous histiocytes protrude shorter tentacle-like pseudopodia, but they have no wandering capacity (Fig. 5). Now, looking at the movement of phagocytes, we find that the higher the species of animals become, the more peculiar and the more active the pseudopodia movement is. Namely, in man (Fig. 6), dogs (Fig. 7), and cats these cells protrude membranous pseudopodia as in the case of monocytes from the entire cell margin and wave these pseudopodia like a flag. Especially in the case of man, their movement can be said to be exactly like monocytes. At a glance the pattern of the movement of ascitic phagocytes indicates that these cells are cells closely related to monocytes, but the wandering velocity of these cells is not so high as that of monocytes. Moreover, similar membranous pseudopodia and other needlelike tentacles can be observed in phagocytes of rabbits (Fig. 8) but their pseudopodia movement is less active; and these cells possess almost no wandering capacity. In the cases of mice (Fig. 9) (Fig. 10) and rats, these cells project pseudopodia, complicated membranous, needle-like or tentacle-like, but their motility is quite much lower than that of higher animals. On the other hand, it is noteworthy that the pseudopodial movement of these cells of chickens is surprisingly similar to the monocytic movement of pseudopodia in the cases of man and dogs. As is clear from these, it seems rational to consider the pattern of movement peculiar to ascitic phagocytes is the same as the D-type movement of monocytes.

Next, according to the phase-contrast microscopic observations on pressure preparations, mitochondria are found around the nucleus and the nuclear membrane is thinner and nuclear network is more minute and these cells resemble more to monocytes rather than to histiocytes. In the neutral red vital staining of the tissue culture neutral red vacuoles are arranged in rosette formation and in higher animals their features correspond closer to that of monocytes. (Fig. 11)

Again looking at the carbon-particle phagocytosis in tissue culture in these cells we observe the particles have a higher tendency to agglomerate (Fig. 12) and also we see no liberation of the particles from the cells with the lapse of time in culture. These findings are also the same as those of mo-

Fig. 11. Relation between Neutral Red granules and Janus Green granules in phagocytes



nocytes. Similarly the higher the species of animals become, the more closely the fluorescence-microscopic findings in tissue culture with acridine orange resemble to those of monocytes.

Summing up these findings, it may be assumed that ascitic phagocytes are not histiocytes but rather they are the cells closely related to monocytes, on the basis of the findings on the pattern of pseudopodial movement as well as those from other vital findings and the positive reaction to peroxidase.

*The origin of ascitic phagocytes* : On the tissue culture of the milky spots of the greater omentum of mice and rabbits (Fig. 13) the wandering cells are exactly the same as ascitic phagocytes, and in addition, most of them are smaller, i. e. younger cells, showing flag-like pseudopodia as well (Fig. 14) when the greater omentum is removed, 5 to 10 days afterward the number of ascitic phagocytes, particularly smaller cells, decrease in number (Tables 3, 4, 5). These data clearly indicate that the source of phagocytes is in the milky spots of the greater omentum. In the study of the embryo of rabbits the milky spots of the greater omentum (Fig. 15) and ascitic phagocytes (Fig. 16) can be recognized already on the 15th

Table 3. 5th day after greater omentum resection

		No. I		No. II		No. III		
		before resect.	5th day	before resect.	5th day	before resect.	5th day	
cell No.		51,700	42,600	61,700	10,000	109,000	42,800	
percentage	phagocyte	total	89.2	48.8	77.4	64.8	86.6	59.2
		large	8.6	11.8	7.6	18.8	5.6	13.2
		medium	60.0	27.6	50.6	36.8	53.8	32.0
		small	20.6	9.4	19.2	9.2	21.2	14.0
	lymphocyte	9.2	2.4	18.4	7.6	6.8	11.2	
	serosa cell	—	—	0.2	0.4	—	—	
	mast cell	1.2	—	0.2	—	0.4	—	
	granulocyte	0.4	48.8	3.8	27.2	6.2	29.6	

Table 4. 10 th day after greater omentum resection

		No. I		No. II		No. III		
		before resect.	10 th day	before resect.	10 th day	before resect.	10 th day	
cell No.		150,300	34,700	109,700	55,600	98,300	72,000	
percentage	phagocyte	total	72.0	72.0	79.2	71.6	84.4	87.0
		large	11.6	4.8	4.4	6.4	2.0	4.6
		medium	46.4	52.8	55.2	48.6	58.8	65.0
		small	14.0	14.4	19.6	16.6	23.6	17.4
	lymphocyte	8.8	5.0	12.8	10.8	10.8	9.4	
	serosa cell	—	0.2	0.4	—	—	—	
	mast cell	—	—	—	—	—	—	
granulocyte	19.2	22.8	7.6	17.6	4.8	3.6		

Table 5. Control (mouse without greater omentum resection)

		No. I			No. II			No. III			
			5th day	10th day		5th day	10th day		5th day	10th day	
cell No.		71,700	80,000	104,700	107,000	152,700	186,300	102,700	118,000	160,300	
percentage	phagocyte	total	88.0	90.4	92.0	89.8	80.8	88.8	80.4	84.4	85.6
		large	9.2	6.2	3.6	11.2	5.0	6.0	6.4	3.4	5.6
		medium	67.2	64.4	77.2	66.8	47.4	66.0	56.0	58.0	73.2
		small	11.6	19.8	11.2	11.8	28.4	16.8	18.0	23.0	6.8
	lymphocyte	10.2	7.8	6.8	6.4	9.6	6.4	17.4	8.4	4.4	
	serosa cell	—	—	—	—	—	—	—	—	—	
	mast cell	1.6	1.0	—	3.0	0.6	1.2	0.2	0.2	0.4	
granulocyte	0.2	0.8	1.2	0.8	9.0	3.6	2.0	7.0	9.6		

embryonic day, and at the same time masses of erythroblasts (Fig. 17) can be observed in the greater omentum. Consequently it can be said that the milky spots after the birth is the remnant of the monocytic cell group that grew during the embryonal stage. The reason why these ascitic phagocytes originating from the milky spots do not have wandering capacity like monocytes in blood seems to lie in the possibility that they might lose their wandering capacity because they proliferate under peculiar circumstances wander- as in the greater omentum or that they might naturally lack in the ing capacity because, suspended in the ascites, they have no necessity of it.

#### CONCLUSIONS

Judging from our vital observation conducted mainly by tissue culture, it was firmly demonstrated that ascitic phagocytes are not histiocytes but



they are the cells closely related to monocytes and that the sites of the genesis are the milky spots of the greater omentum. The milky spots are most possibly the remnants of the mesenchymal hematopoiesis of the embryonic stage.

#### ACKNOWLEDGEMENT

The authors express their deep gratitude to Prof. Hiraki for his guidance throughout this study.

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LEGENDS FOR FIGURES

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- Fig. 2. Ascitic phagocyte of dog (M. G. staining).
- Fig. 3. Ascitic phagocytes of mouse on irritation (M. G. staining).
- Fig. 4. Human monocytes in blood (PCM-BM).
- Fig. 5. Subcutaneous histiocytes of mouse (PCM-BM).
- Fig. 6. Human normal ascitic phagocytes (PCM-BM).
- Fig. 7. Ascitic phagocytes of dog (PCM-BM).
- Fig. 8. Ascitic phagocytes of rabbit (PCM-BM).
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- Fig. 13. Milky spot of greater omentum cultivated for 6 hours (mouse).
- Fig. 14. Milky spot cells cultivated for 76 hours (rabbit).
- Fig. 15. Milky spot of 25th embryonic day (rabbit).
- Fig. 16. Ascitic phagocytes of 19th embryonic day (rabbit).
- Fig. 17. Erythroblasts of 25th embryonic day (rabbit).

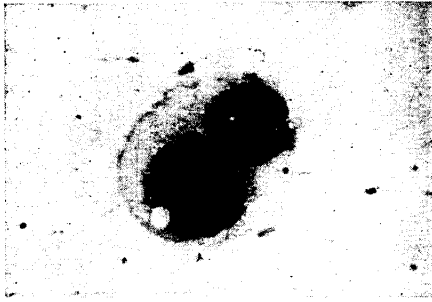


Fig. 2.



Fig. 3.



Fig. 4.

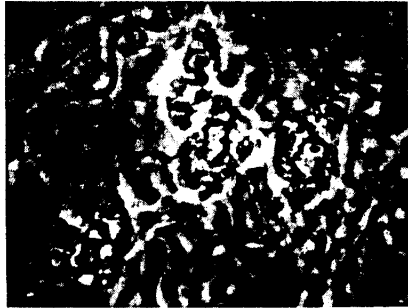


Fig. 5.



Fig. 6.

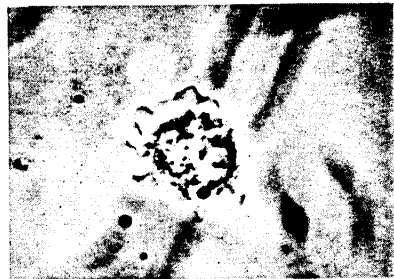


Fig. 7.



Fig. 8.

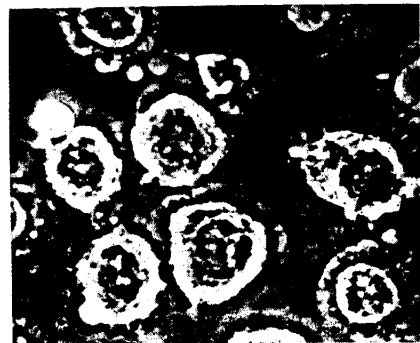


Fig. 9.

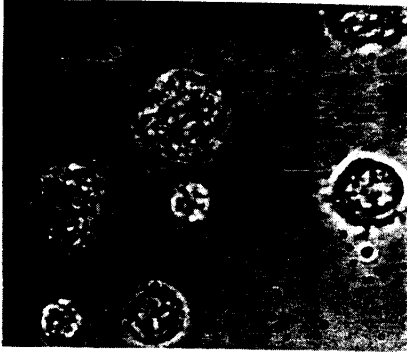


Fig. 10.

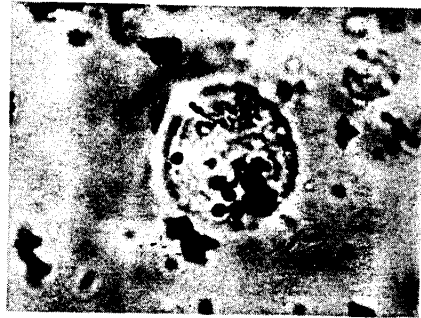


Fig. 12.

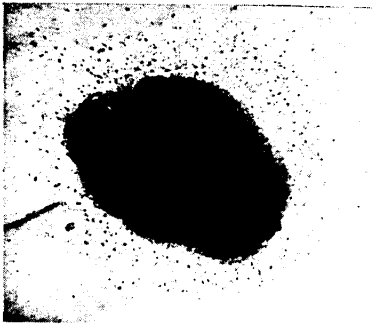


Fig. 13.



Fig. 14.

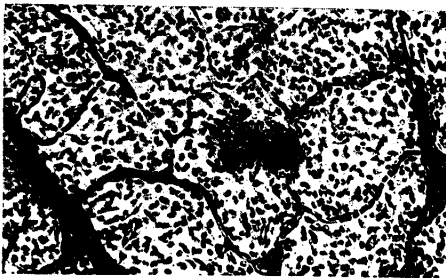


Fig. 15.

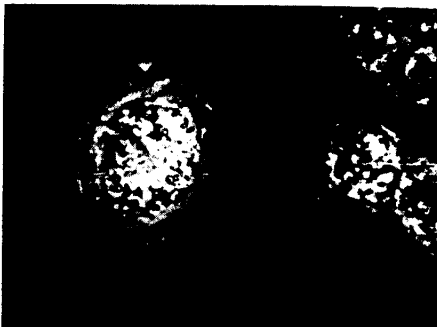


Fig. 16.



Fig. 17.