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A STUDY OF PHAGOCYTOSIS IN FREE CELLS OF THE COELOMIC FLUID OF NEWTS, *TRITURUS PYRRHOGASTER*

By

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Introduction

Among the free cells native to the coelomic cavity of newts, the characteristic morphology of a cell with Russell's bodies (so-called Russell's cell) has been of special interest, and some cytological studies have been undertaken by Ohuye (1936) and Mizutani and Nakahara (1961). Recently, Seto (1970a, 1970b) has reported on certain cytological features of the free cells under experimental conditions with special regard to the Russell's cells. According to his observations, lymphocytes and monocytes in the coelomic fluid of newts showed positive responses to such mitotic initiating agent as Phytohemagglutinin (PHA-M) or to ^{3}H-Thymidine indicating the occurrence of DNA synthesis at interphase, while Russell's cells showed no predominant multiplicity even after the treatment of a mitotic initiating agent nor active DNA synthesis except for the young cell. However the Russell's cell in the coelomic fluid varies in number over a wide range with the individual animal, and even after the aspiration of a large number of Russell's cells from the body cavity the animal could soon replace those cells lost. The reason for this phenomenon would be explained by answering the following questions : (1) How is the Russell's cell formed in the body cavity, and (2) what kind of function does the cell have *in vivo*.

Therefore it is necessary to clarify the origin and the function of the Russell's cell in a body cavity. Since phagocytotic activity, which is revealed by ingestion of carbon particles of the India ink, was regarded as an important cellular function (De Robertis *et al*. 1965), the present experiment makes it possible to compare a cell's function among coelomic free cells in order to know the similarity or dissmilarity of the Russell's cell to other free cells.

Materials and Methods

Adult male and female newts, *Triturus pyrrhogaster*, collected in the vicinity of Matsue, have been used in the experiment. Animals were stocked in a plastic water tank (32×50×12 cm) throughout the experiment and regularly fed liver fragments from a pig once a week.

Newts were injected intraperitoneally with 0.4 ml of the India ink diluted 1 : 25 with amphibian Ringer solution. The diluted ink was filtrated once through No. 1 filter paper (Toyo Roshi, Ltd.) before use. The carbon particles of the India ink (The

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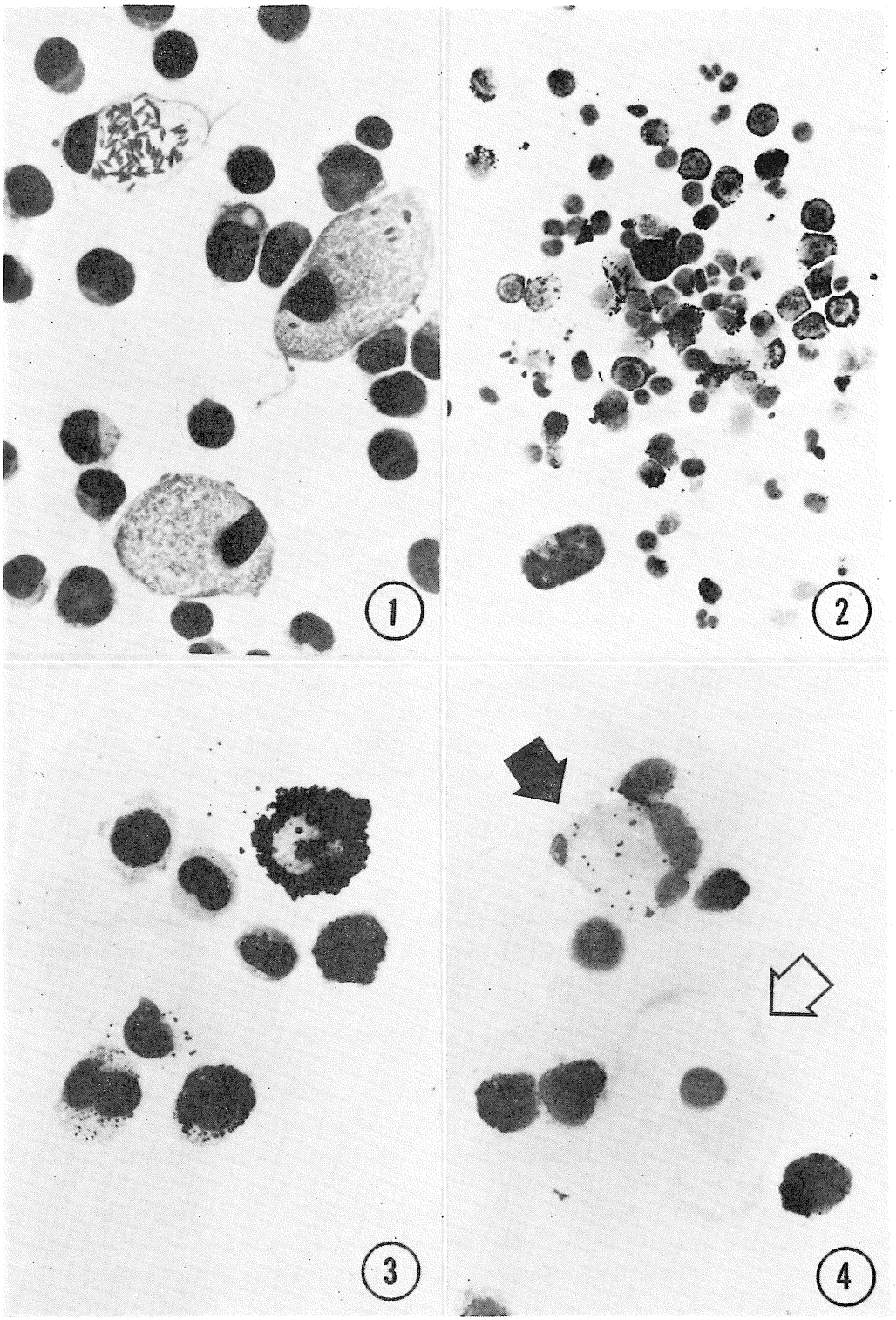
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Japanese Sumi ; Kuretake K. K.) were fine grained enough to be intaken by the cells and easy to find in the cytoplasm after the cytological preparations of fixation and staining.

After a single inoculation of the India ink into the newt body cavity, free cells in the coelomic fluid were taken out once every seven days with a glass capillary pipette and prepared for the cytological examinations. This sampling was repeatedly undertaken 12 times and free cells were examined by the smear preparation, following the procedures of methanol-fix and May-Grünwald Giemsa stain as described before (Seto 1970a).

Results

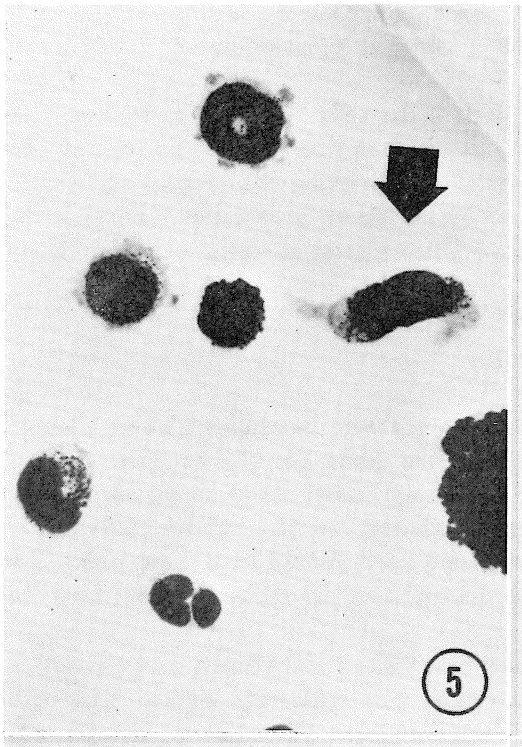
At the beginning of the observation these animals were ascertained as to whether or not the treatment of free cells with 0.4 ml of the India ink of a 1 : 25 dilution in the Ringer was successfully inoculated into the body cavity. Four out of six animals were continuously used in the experiment to observe the phagocytotic activities of coelomic free cells *in vivo*. The other two animals were discarded from the experimental group due to unsuccessful injection that was distinguished by ink leaking out from the body cavity.

Twenty-four hours after intraperitoneal injection, the ink still remained in the coelomic fluid staining it a darkish color. Free cells were selectively stained black as a result of absorbing carbon particles from the ink (Fig. 2). The most remarkable absorption of carbon particles was done by monocytes and neutrophilic granulocytes. Granulocytes were easily recognized by their nuclear shape, and their cytoplasm was found to be fully occupied with black particles. Monocytes consisting of various sizes, usually the largest element among white blood cells, consistently contained lots of carbon particles in cytoplasm. Lymphocytes, both of large and small types, showed phagocytotic activity but their cytoplasm contained a smaller amount of carbon particles than monocytes at the beginning of the observation. Eosinophilic granulocytes, which were in the minority but were easily identified by the appearance of reddish granules detected with May-Grünwald stain, never absorbed carpon particles into their cytoplasm. There was no Russell's cell which revealed phagocytotic activity at 24 hours after the treatment although some carbon particles were occasionally attached to the cell surface.

(*Explanations of Figures*) Photomicrographs of free cells in the coelomic fluid of newts before and after the treatment of the diluted India ink. All pictures were taken from the smear preparation, methanol-fix, and May-Grünwald Giemsa stain.

Figure 1. The coelomic free cells of an untreated newt, consisting of monocytes, granulocytes, lymphocytes and Russell's cells.

Figures 2-4. The coelomic free cells after the inoculation of a dilution of the India ink. The cells which ingested the India ink contain carbon particles in their cytoplasm. 2, a general view of a group of the free cells from a newt at 48 hours after the treatment. ×180. 3 & 4, the free cells of 7 days after the treatment, which showing phagocytotic activities in monocytes and macrophages in contrast with the mature type of Russell's cell (*arrow*). The young type of the cell occasionally bearing a few carbon particles (*arrow*). ×480.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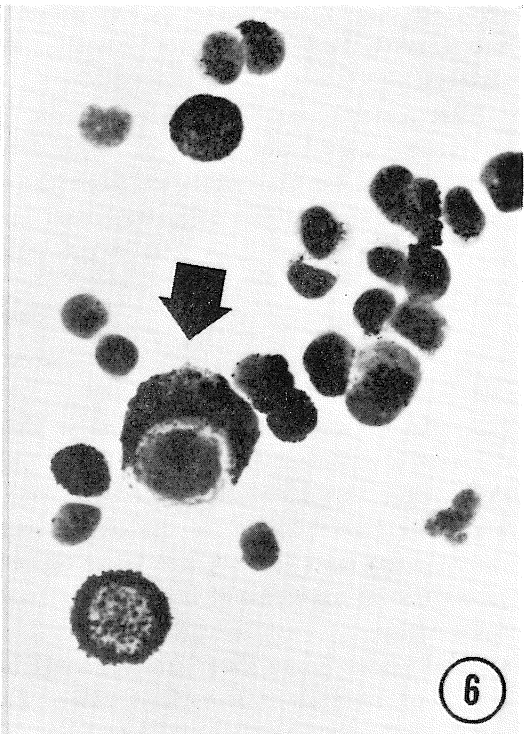
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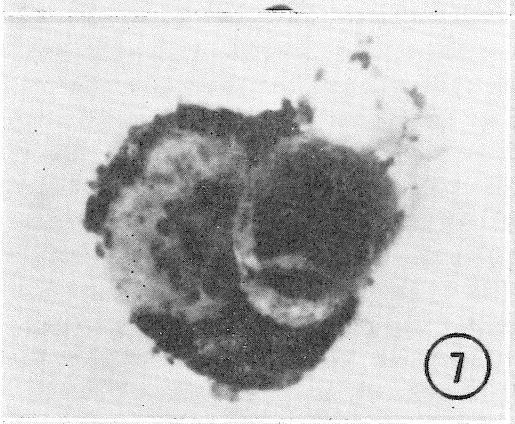
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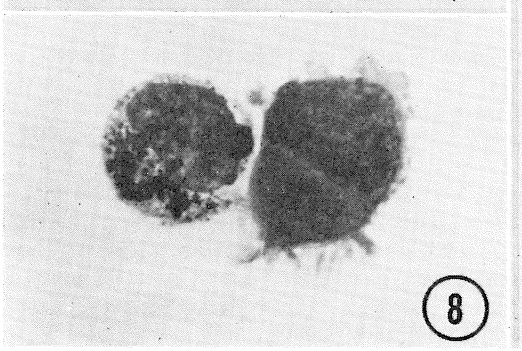
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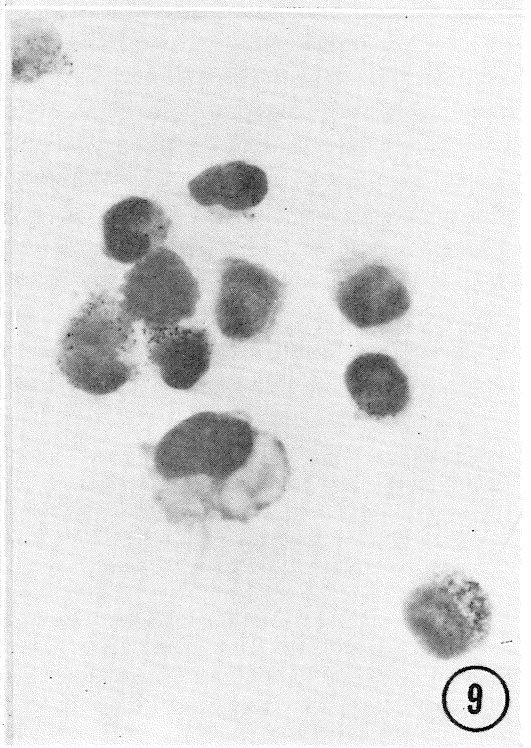
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Seven days after the inoculation, first-week specimens were examined from each animal. Large lymphocytes, monocytes and neutrophilic granulocytes greatly absorbed the carbon particles. The picture of the reaction of Russell's cells was specially marked. The mature Russell's cells, which were classified by the size of vacuoles and well-developed protoplasmic processes (Fig. 1), hardly showed any ingestion of carbon particles, while the young cells did lightly ingest the carbon during the first week (Fig. 3 & 4).

As shown in Table I, an interesting observation was made after second week concerning the Russell's cells. Larger elements, recognized as a mature type of Russell's cell containing an increased amount of carbon particles, began to appear at the second week's end. In specimens taken 14 days after the injection, the number of Russell's cells which contained carbon particles increased to twice the number of carboon-bearing cells found after the first week. Then, the number of Russell's cells bearing carbon particles showed an ever greater increase (Figs. 5 & 6). The maximum degree of appearance of carbon particles in the cells occurred at the end of the third week (21 days after inoculation). At this time in about 47 per cent of the Russell's cells were detected black grains in their cytoplasm. As these carbon particles were not directly ingested by mature types of Russell's cells but absorbed by the young types, the fact that progressive transition had been occurring during those weeks from the young types to the mature types was indicated (Figs. 7 & 8).

In the 4th week, the number of the carbon-bearing Russell's cell began to decrease (Fig. 10). Then a notable disappearance of the cell successively took place in the 7th to 8th weeks. This drastic decrease in number of cells would imply that progressive decline of mature types of Russell's cells occurred within the 2 months. In contrast with the decrease in the number of carbon-bearing Russell's cells, newly produced monocytes, which have no carbon particles, appeared and progressively increased in number during these weeks (Fig. 9). Subsequently the young type of Russell's cells, which did not ingest carbon particles, continued to multiply. Then the carbon-bearing Russell's cells of the mature type found in the coelomic fluid became relatively scarce. However a few of the mature cells, about less than one per cent of the Russell's cells, maintained various amouns of carbon particles throughout the test tperiod of 84 days.

Figures 5-9. The coelomic free cells after the inoculation of a dilution of the India ink. The cells bearing carbon particles in the cytoplasm indicate the ingestion of the India ink after the treatment except for a mature type of Russell's cell. 5 & 6, the free cells in the third weeks after the treatment. A young type of Russell's cell (*arrow*) contains carbon particles as well as monocytes. × 480. 7, a Russell's cell in the 4th week after the treatment bearing carbon particles. These particles were not directly intaken by the Russell's cell but the result of cellular transformation from the ingested cell. × 800. 8, a young type of Russell's cell lightly contains carbon particles in contrast to a monocyte, in the 3rd week. × 800. 9, free cells in the 6th week after the treatment. Newly produced monocytes do not contain carbon particles and number of grains in ingested cells getting decrease. Carbon-bearing Russell's cell is hardly found in the preparation. ×480.

TABLE I. Changes in proportion of Russell's cells bearing carbon particles after single injection of diluted India ink.

Weeks after injection	Animals examined	No. of R-cells* observed (A)	No. of R-cells* intook carbon particles (B)	Per cent	Total number (A)	Total number (B)	Average (per cent)
1	I	100	14	14.0	272	47	17.9
	II	45	7	15.6			
	III	27	6	22.2			
	IV	100	20	20.0			
2	I	85	36	42.4	213	87	40.1
	II	6	2	33.3			
	III	22	11	50.0			
	IV	100	38	38.0			
3	I	100	52	52.0	183	87	47.5
	II	33	10	30.3			
	III	35	18	51.4			
	IV	15	7	46.7			
4	I	100	45	45.0	247	86	34.8
	II	88	22	25.0			
	III	25	10	40.0			
	IV	34	9	26.5			
5	I	100	28	28.0	349	111	31.8
	II	73	26	35.6			
	III	76	28	36.8			
	IV	100	29	29.0			
6	I	67	20	29.9	322	78	24.2
	II	65	11	16.9			
	III	90	29	32.2			
	IV	100	18	18.0			
7	I	60	18	30.0	307	54	17.6
	II	47	8	17.0			
	III	100	16	16.0			
	IV	100	12	12.0			
8	I	43	4	9.3	266	14	5.3
	II	23	0	0			
	III	100	9	9.0			
	IV	100	1	1.0			
9	I	36	2	5.6	151	5	3.3
	II	15	2	13.3			
	III	50	1	2.0			
	IV	50	0	0			
10	I	100	2	2.0	281	2	0.7
	II	36	0	0			
	III	100	0	0			
	IV	45	0	0			
11	I	57	1	1.8	244	2	0.8
	II	48	0	0			
	III	100	0	0			
	IV	39	1	2.6			
12	I	100	4	4.0	364	5	1.4
	II	91	0	0			
	III	73	1	1.4			
	IV	100	0	0			

* The mature type of Russell's cells.

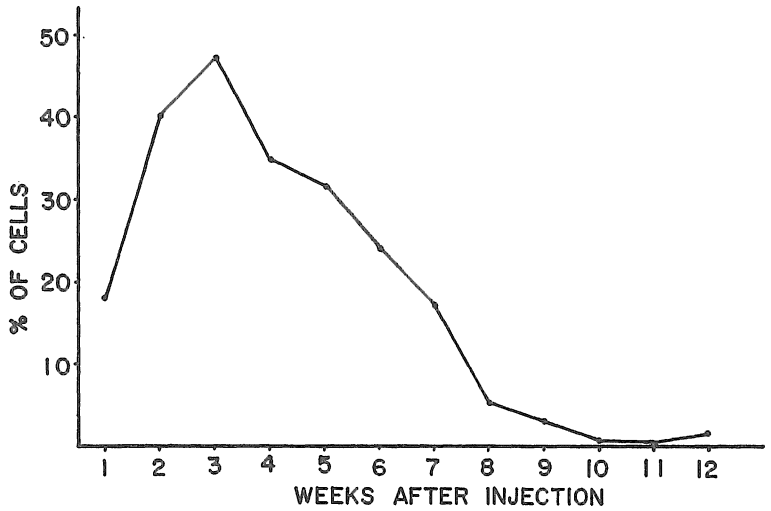

Figure 10. Appearance of Russell's cells bearing carbon particles in the cytoplasm after single intraperitoneal injection of diluted India ink. Russell's cells here observed were limited to the mature type.

Discussion

From the results of previous experiments of the Phytohemagglutinin treatment on the free cells, the author (Seto 1970a) supposed that the Russell's cell might be derived from a monocyte or a large lymphocyte by means of cellular transformation. The present investigation of cell function as represented by a phagocytosis may supply some evidences for this suppositive connection between Russell's cells and white blood cells.

Phagocytosis is intimately related to the activity of the plasma membrane as well as pinocytosis. In some cases, the cell may actively ingest large, solid particles, and then the process of penetration generally is visible with the light microscope. In Metazoa, phagocytosis has a great importance in pathologic conditions as a general defense mechanism of the organism (De Robertis *et al.* 1965). Therefore it is meaningful as an indication of cellular function to compare the phagocytotic activity of Russell's cells to other types of free cells derived from hematopoietic organs.

The present experiment clearly showed that phagocytosis was highly developed in neutrophilic granulocytes and in the cells of the macrophagic or reticuloendothelial system. The coelomic free cells are mostly monocytes and lymphocytes belong to this group which includes histiocytes of connective tissue. However, the Russell's cells have not been proved to belong to the group of reticulocytes or macrophages, nor is their function understood.

As shown above, the mature type of Russell's cells bearing carbon particles were found during the first to second week after the India ink injection. But they did not show any direct ingestion of carbon particle, especially immediately after the injection.

Therefore this notable increase in number of carbon-bearing Russell's cells observed in the third week indicates the young types of Russell's cells or other types of free cells which ingested carbon particles could have matured or transformed into the mature type cells. Such transition was supposed in the previous study of DNA synthesis in free cells in the coelomic fluid *in vivo*, indicating that only monocytes, lymphocyets, and young type of Russell's cells were proved to have proliferative ability.

Similar facts have been observed by Felix and Dalton(1955). In their morphological study of peritoneal free cells of mice they noticed large numbers of cells in transition between lymphocytes and macrophages in the body cavity. These cells also closely followed in form the descriptions of Bloom (1928, 1938) in his studies of the transition of lymphocytes through macrophages to fibroblasts in tissue culture. Therefore a reasonable explanation for the increase in number of mature type of Russell's cells could be made that cellular transformation from other type of free cells was occurred; it could have been transformed from monocytes or lymphocytes through the young types of Russell's cells. Because these cells maintain the proliferative ability and active phagocytotic activities in the body cavity. More intensive studies of the cell function are required to conclude this problem.

Summary

1. In order to observe a certain function of the Russell's cell as well as other free cells in the coelomic fluid of newts, phagocytotic activities were induced by means of single inoculation of diluted India ink into the body cavity.

2. Active phagocytotic activity was observed in monocytes, large lymphocytes, neutrophilic granulocytes and macrophages in the first week of the treatment.

3. Although Russell's cells did not ingest carbon particles of the ink, carbon-bearing Russell's cells gradually appeared in the preparations of the first week, and then the number increased at the third week.

4. There was a notable decrease in appearance of the carbon-bearing Russell's cells thereafter. This phenomenon would indicate that progressive decline of mature type of Russell's cells occurred within 2 months.

5. From the observation of all types of free cells which ingested the carbon particles and the results of the investigation of changes in number of carbon-bearing Russell's cells supported the supposition of a possible cellular transformation of Russell's cells from monocytes or lymphocytes through the young type of Russell's cells.

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