THE ORIGIN AND FATE OF TWO TYPES OF MULTINUCLEATED GIANT CELLS IN THE CIRCULATING BLOOD

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PLATES 10 AND 11

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In the course of studies in the experimental production of giant cells in the tissues, reported by Doan, Sabin and Forkner (1), the writer discovered a procedure by which giant cells could be made to appear in the blood stream. This new material gave valuable evidence concerning the formation of giant cells, more especially of those of the so called foreign body and epithelioid types.

As far as can be ascertained, no one has hitherto reported a method to induce an appearance of giant cells in the blood, and no such cells have been found there, except megakaryocytes, which were reported by Oelhafen (2) and Naegeli (3) in 1914, by Minot (4) in 1922, by Sabin (5) in 1923, and by others. The usual condition in which they have been found is myelogenous leucemia.

Haythorn (6), in a recent review, has classified giant cells as follows: (a) Langhans' giant cells, (b) foreign body giant cells, (c) osteoclasts, (d) megakaryocytes, (e) muscle giant cells, (f) giant cells of nervous tissue, and (g) true tumor giant cells. It is relative to the first two of these types that this paper is concerned.

As early as 1868 Langhans (7), working with fresh tissues, demonstrated large multinucleated cells obtained from tuberculous lesions. For the most part the nuclei were arranged around the periphery of the cell, were usually round or oval, had sharp outlines and generally contained nucleoli. He stated that the protoplasm of such a giant cell was pale, homogeneous, or finely granular, with the center usually clear. This type of cell has since been designated the "Langhans' giant cell." However, some confusion has arisen because he also described another type of giant cell which had nuclei distributed throughout the cytoplasm.

For reasons given by Doan, Sabin and Forkner (1), the term *"epithelioid giant cell"* has been proposed to replace the often misunderstood term of Langhans' giant cell. This concept of the "epithelioid giant cell" is based on new information obtained through the methods of studying living cells with supravital (Simpson, 8)

(Sabin, 5) and vital stains. It was first demonstrated by Sabin, Doan and Cunningham (9) that the epithelioid cell is derived from the monocyte. The supravital technique showed that in the monocyte, surrounding its centrosphere, is a characteristic rosette of vacuoles, stainable in the living state with neutral red. An accentuation of this normal structure of the monocyte produces an epithelioid cell. Cunningham, Sabin, Sugiyama, and Kindwall (10) then confirmed the findings of other investigators that the Langhans' giant cell is a multinucleated epithelioid cell.

In general there have been two opinions concerning the types of cells under discussion. One group of investigators, among them Ziegler (11), Kockel (12), Hektoen (13), Maximow (14), and Medlar (15), believe that the Langhans' giant cell is merely a foreign body giant cell occurring in tuberculosis. Krückmann (16) studied giant cells in tuberculosis, about parasites and foreign materials, and in tumors. He concluded that microscopic means were not available by which foreign body giant cells and Langhans' giant cells could be distinguished. Langhans (7), Jacobson (17), Lubarsch (18) and others, however, believe that the giant cell of tuberculous tissue can be distinguished from all other kinds.

Concerning the mode of origin of the foreign body and epithelioid giant cells there has been some disagreement. Weigert (19), Baumgarten (20), Bakács (21), Lubarsch (18), and others were of the opinion that giant cells of tuberculous lesions were formed by continued nuclear division. Krauss (22), Mallory (23) and Wells (24) are among those who favor fusion of individual cells as the explanation for the formation of the types of cells under discussion. Lewis and Webster (25) in 1921 believed that epithelioid giant cells were formed by amitosis of the nucleus, but a later paper by Lewis (26) takes the opposite view, that they are formed by fusion of epithelioid cells.

Materials and Methods

The experiments reported in this communication have been carried out with the use of the methods of supravital and vital staining. Because of some recent minor modifications in the methods used in Dr. Sabin's laboratory, the solutions of dyes employed and their method of preparation will be described in detail.

We are now using six different solutions for our routine work. Solution 1 is prepared by dissolving 125 mg. of vital neutral red (Grübler) in 50 cc. of neutral absolute ethyl alcohol. This makes a 0.25 per cent solution which is saturated. It may be stated here that neutral red (Ehrlich) certified for use in vital staining as prepared by the National Aniline and Chemical Company, New York, is a thoroughly satisfactory dye which can be substituted for neutral red (Grübler). Solution 2 is prepared by dissolving 125 mg. of vital Janus green (National Aniline or Grübler) in 62.5 cc. of neutral absolute ethyl alcohol, thus making a 0.20 per

cent solution which likewise is saturated. By adding 50 drops of Solution 1 to 10 cc. of neutral absolute ethyl alcohol, we prepare Solution 3. Solution 4, a mixture of neutral red and Janus green, is made by adding 2 drops of Solution 2 to 3 cc. of Solution 3.

For the study of blood or other tissues, where cells to be stained are relatively few in number, we prepare slides in the manner described by Sabin (5). This is accomplished as follows: (a) by means of a pipette, flood the upper surface of a slide chemically clean with Solution 4; (b) drain excess of dye back into bottle by holding slide in an upright position; (c) absorb the excess of dye on the dependent edge of the slide with blotting material; (d) evaporate the remaining alcohol on the slide by immediately holding it over a piece of wire gauze under which a gas flame is burning. There is no objection to burning the excess of alcohol from the slide. The stained surface of the slide is then marked with a wax pencil and the slides are stored for use. Some investigators in the past have experienced difficulties in obtaining an even distribution of the dye over the slide, particularly when there is a high degree of humidity in the atmosphere. Preparing the slides as described, in artificially dried air, obviates these troubles, leaving a thin, even film of the dye.

For study of bone marrow, lymph nodes, or other tissues where there is an abundance of colorless cells to be stained, two more solutions are required. Solution 5 is prepared by adding 150 drops of Solution 1 to 10 cc. of neutral absolute ethyl alcohol. Solution 6, a mixture of neutral red and Janus green, is made by adding 8 drops of Solution 2 to 3 cc. of Solution 5. Thus Solution 6 contains approximately three times the amount of the dyes as Solution 4. Slides are prepared with Solution 6 in precisely the same manner as with Solution 4. The latter solutions, 4 and 6, being mixtures of neutral red and Janus green, deteriorate after about 24 to 48 hours and must be freshly prepared each time they are needed. The remaining solutions are stable and are best kept in glass stoppered bottles in a cool, dark place. Care must be exercised to make sure that the pipettes and bottles used are free of alkali. If an excess of alkali is present, the neutral red solution will become a muddy yellow color.

Blood films in these supravital studies are prepared by obtaining a drop of blood on a clean coverslip and letting it fall gently on the slide prepared from Solution 4. The film is quickly rimmed with vaseline (salvoline) and in the course of from 3 to 5 minutes is ready for study. The films are best studied in a constant temperature box at 38° or 39°C. For the study of the cells of organs such as the omentum or subcutaneous tissues, thin films of the intact tissue may be spread over slides prepared with Solution 6. If the organs are too dense or too thick to be spread intact over a slide, the cut surface of the fresh tissue, for example, lung, lymph nodes, etc., is scraped with a sharp scapel and the tissue accumulating on the knife blade is placed on a slide prepared from Solution 6. If the tissue is very dry a drop of plain normal salt solution or normal salt solution moderately colored with neutral red may be added to suspend the cells. A coverslip is then applied as above and is rimmed with vaseline. Material prepared in this way provides a thin enough film so that the individual living cells can be studied. For our

purposes, tissues fixed in Helley's fluid, embedded in paraffin, and stained by the usual methods were used to supplement the supravital studies.

The fundamental experiments dealing with giant cells were carried out on the blood and tissues of rabbits which had been injected with various substances, namely, agar, lycopodium spores, paraffin, olive oil, mineral oil, living tubercle bacilli, and certain lipoid fractions isolated from tubercle bacilli. These latter substances were obtained from Dr. R. J. Anderson of the Sterling Chemical Laboratories of Yale University, through the Research Committee of the National Tuberculosis Association. A full account of these reactions has been presented by Doan, Sabin and Forkner (1). None of the substances studied produced giant cells in the circulating blood, except agar. Consequently the development of this special phase of the giant cell study is here presented separately.

Experimental Data

The early experiments in which relatively small amounts of agar were introduced into the tissues produced no significant changes either quantitative or qualitative in the cells of the blood. However, since the local tissue response was so profound such large numbers of monocytes and giant cells having been produced, the possibility presented itself that a larger and more disseminated foreign body reaction might carry over the tissue response to the blood. This theory seemed to have merit since it was known from experience in this laboratory that certain diseases, notably tuberculosis, are often associated with the finding of pathologic tissue elements, epithelioid cells, in the circulating blood.

Accordingly, an animal, R 790, was injected, on February 25, 1929, with plain sterile agar in many areas under the skin and intraperitoneally.

A total of 40 cc. of the agar at pH 7.4 was introduced. Incidentally this animal was given frequent intravenous injections of trypan blue in order that the reactions of the giant cells of the tissues to this vital dye might be studied. Their responses are discussed elsewhere (1). Let it suffice to say that the trypan blue did not materially influence the blood picture in this animal, except that it may have been responsible for the increased number of macrophages.

The blood had been examined prior to the injections, but was not studied again

until 1 week later (March 4). At this time there was a slight reduction of the hemoglobin and number of red corpuscles. The total white blood cell count was not changed. Monocytes were distinctly high (21 per cent) and the lymphocytes were lower than they had been in the control period. The blood platelets were clustered in large masses. Some individual blood plates were larger than normal. The platelets were undoubtedly greatly increased in number. The blood contained 11 per cent of clasmatocytes (macrophages) which were usually large cells with single, centrally placed nuclei. Their cytoplasm contained débris of various shades of color and various sizes and shapes. The presence of the large number of these cells on this occasion may be explained as the result of the injection of trypan blue. Such phenomena have been studied and reported by Simpson (8). The character of the monocytes on this and subsequent days was of great interest. Many of them were much larger than normal, possessed round or oval nuclei, often with two or three nucleoli, and each cell contained a rosette of exceedingly fine uniform neutral red bodies. In some instances the rosettes were small, whereas in others they were hypertrophied, making a stimulated monocyte which is a transition between a normal monocyte and an epithelioid cell. These forms are entirely similar to the developing monocytes which Forkner (27) has described and illustrated as normal elements in peripheral lymph nodes of rabbits. Another interesting change was noted in the polymorphonuclear neutrophils. Many of these cells had a decreased number of specific granules and contained more neutral red bodies than are normally present. These neutral red bodies normally are round or globular whereas after agar injections the same bodies are present and in addition many larger, often elongated, non-refractive vacuoles or granules.

Besides these changes in the usual blood cells, typical multinucleated giant cells could be found in every smear on this and subsequent days until the animal was autopsied on March 18, 1929, 3 weeks after the agar injection. The photograph of one of these cells is illustrated in Fig. 11. This cell had about 25 nuclei scattered through all parts of the cytoplasm. There was an area near the center of the cell which was probably agar. It was a homogeneous amorphous material which had a purple tint. It had apparently taken on some of the trypan blue as the agar masses in the tissue had done. The nuclei near the periphery tended to be oval, whereas the others were round in shape. Individual nuclei were quite uniform in size and were somewhat larger than red blood corpuscles. Some of them contained nucleoli. The cytoplasm contained scattered, irregular masses of material staining all shades of red, yellow, and brown. There was no pattern to the arrangement of the material stained with neutral red.

Fig. 9 represents a cell from the peripheral blood of this animal. A coarse mass of agar is seen to lie across the center of the cell and on either side are segregation bodies entirely similar to those encountered in epithelioid cells and monocytes. One can, in fact, make out two areas in which the arrangement of the vacuoles suggests rosettes of neutral red bodies in the upper part of this cell, indicating a possible fusion of two or more mononuclear or polynuclear cells around the large

mass of agar. This last cell (Fig. 9) is difficult to classify in one or the other group, but on the whole it is more like a foreign body giant cell.

Another interesting group of cells from Rabbit R 790, shown in Fig. 4, illustrates how the giant cells may be formed or how they may be disintegrated. In this figure are three stimulated monocytes which have fused together about a blue mass of agar. When first seen this group had an almost spherical cell membrane surrounding the three cells and the agar. They were observed in the warm box for about 2 hours and were seen to separate into three distinct living cells with only a thread of cytoplasm connecting them. The agar was left free between them. This phenomenon may represent the fate of foreign body giant cells by disintegration into separate uninuclear cells. Other instances have been observed where giant cells of the foreign body type could be seen to be composed of many individual monocytes with other monocytes or epithelioid types nearby, apparently fusing to form a giant cell (Figs. 12 and 13). It would appear then that this is evidence similar to that recorded by Hektoen (13). He studied healing, nondegenerative tuberculous tissue of the brain and found that many giant cells are disintegrated into mononuclear cells which retain their function. It is not clear from his text whether the giant cells which he observed as separating into mononuclear elements were according to the classification here employed "epithelioid giant cells" or foreign body giant cells. I have found no evidence for this phenomenon in "epithelioid giant cells." Later Hektoen (28) supported his former view by observing the same phenomenon in the fate of the giant cells which form in the absorption of coagulated blood serum, in the anterior chamber of the rabbit's eye. In these latter experiments it seems that he was probably dealing with the foreign body type of giant cells.

Rabbit R 579 was injected with agar as the previous animal had been, but no dye was introduced.

A preliminary study of the blood before the injection showed a normal formula. There were 7,600 white blood cells and 5,250,000 red blood cells per cubic millimeter. There were 11 per cent monocytes. 2 days after the injection of agar, the monocytes rose to 20 per cent and increased in total number from 836 to 1,580 per cubic millimeter. 6 days after the injection, monocytes became more numerous than any other cell, increasing to 49 per cent or 5,120 cells per cubic millimeter. A chart (Chart 1) makes this reaction clear. In this experiment, there was a reciprocal relationship between the numbers of monocytes and lymphocytes. As in the preceding experiment, many of the monocytes were of the young type, larger than the adult cells, possessing round or oval nuclei with nucleoli and having an extraordinary abundance of delicate mitochondria. Some of the mature monocytes contained coarse irregular brownish bodies in their cytoplasm, which were probably pieces of agar undergoing digestion.

Giant cells were seen in the blood on the eighth and ninth days. On the eighth day enough were present to be recorded in the differential count on a percentage

basis; on the ninth day, only an occasional cell in an entire film, indicated on the chart by a + sign, was seen. One of these was drawn (Fig. 6). It is clearly of the foreign body type with scattered nuclei and abundant irregular masses of material in the cytoplasm, probably agar undergoing fragmentation and disintegration. The nuclei contained definite nucleoli. Mitochondria were present and easily seen near the periphery of the cell. Adjoining this cell but not fused with it is a monocyte containing the same kind of material as in the giant cell. It is apparent that the larger cell might easily be considered as representing a fusion of many of the individual monocytes. Another cell was drawn (Fig. 7) which has a central rosette and three peripheral nuclei. The material in the cytoplasm is

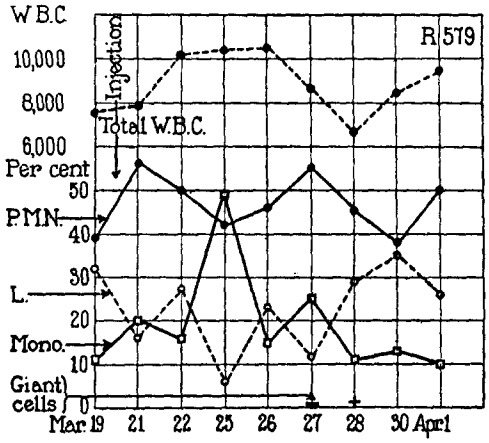

CHART 1. Graph of the white blood cells of Rabbit R 579. The arrow indicates the time at which 40 cc. of sterile plain agar was given in many subcutaneous areas and intraperitoneally. For giant cells the symbol + means the presence of an occasional giant cell in an entire preparation; the solid blocks indicate their percentages when sufficient to appear in the differential count.

not typical of an epithelioid cell. It looks much more like a small foreign body giant cell. It is impossible to say whether this giant cell has arisen by fusion of independent elements or has formed by division of the nuclei. Fig. 8 represents a small foreign body giant cell of the same type as Fig. 7, but containing much coarser bodies. The nuclei are flattened out against the cell wall. This cell, together with that shown in Fig. 7, illustrates that one can not always identify this type of giant cell by the peripheral arrangement of the nuclei. In order to differentiate them properly one must study all of the cellular organs. The presence of a central rosette of fine neutral red bodies aids materially and is probably the most important single factor in the separation of "epithelioid giant cells" or the so-called Langhans' cell. The lungs of this and the other animals were studied in

TABLE I

Changes in the Blood Cells as the Result of Agar Injections into the Tissues

Experiment	Date	White blood cells per cu. mm.	Red blood cells per cu. mm.	Neutrophils		Basophils		Eosinophils		Monocytes		Lymphocytes		Giant cells		Clasmatocytes		Unclassified		Hemoglobin per cent
				Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	
R 790	1/12/29	4,850	5,490,000	45	2,182	5	242	1	48	11	533	38	1,843							68
	1/14	6,400	5,800,000	30	1,920	14	896	2	128	7	448	47	3,008							65
	2/25	6,250	6,200,000	56	3,500	8	500	1	63	7	438	28	1,750							76
	2/25	Began frequent intravenous injections of trypan blue. Injected 40 cc. of agar intraperitoneally and subcutaneously.																		
	3/4	5,500	4,580,000	52	2,860	6	330			21	1,155	10	550		+	11	605			57
	3/6	4,100	4,000,000	58	2,378	14	574	1	41	6	246	18	738		+	3	123			56
	3/7	7,500	4,140,000	63	4,725	10	750	1	75	2	150	22	1,650		+	1	75	1	75	55
	3/9	3,800	4,360,000	62	2,356	12	456	1	38	5	190	19	722		++					49
	3/11	4,200	4,940,000	72	3,024	1	42	2	84	1	42	23	966		+	1	42			56
	3/12	7,950	4,480,000	72	5,724	2	159	2	159	3	238	21	1,669		+					61
	3/15	8,300	3,790,000	68	5,644	18	1,494	1	83	4	732	8	664		+	1	83			53
	3/18	7,250	4,690,000	53	3,842	22	1,595			8	580	13	942		+	2	145	2	145	62
R 579	3/19	7,600	5,260,000	39	2,964	17	1,292	1	76	11	836	32	2,432							
	3/19	Injected in 6 subcutaneous areas intraperitoneally with 40 cc. of plain agar.																		
	3/21	7,900	4,840,000	56	4,424	5	395			20	1,580	16	1,264					3	237	52
	3/22	10,200	4,540,000	50	5,100	4	408	2	204	16	1,632	27	2,754					1	102	55
	3/25	10,450	5,260,000	42	4,389	1	104			49	5,120	6	627					2	209	64
	3/26	10,550	5,210,000	46	4,853	10	1,055	4	422	15	1,582	23	2,426					2	211	59
	3/27	8,700	5,320,000	55	4,785	3	261			25	2,175	12	1,044	1	87			4	348	64
	3/28	6,700	4,620,000	45	3,015	12	804	2	134	11	737	29	1,943	++	++			1	67	58
	3/30	8,450	4,520,000	38	3,211	13	1,098	1	85	13	1,098	35	2,957							
	4/1	9,500	4,790,000	50	4,750	12	1,140	2	190	10	950	26	2,470							55

R 580	3/19	11,000	4,280,000	37	4,070	9	990	1	110	9	990	43	4,730					1	110	60
	3/19	4 cc. trypan blue every 2nd day and 40 cc. plain agar injected intraperitoneally and subcutaneously today.																	
	3/21	45,500	4,440,000	52	23,608	4	1,816			37.5	17,025	4.5	2,043		+			2	908	52
	3/22	21,900	4,630,000	53	11,607	1	219			40	8,760	4	1,314							65
	3/23	42,850	4,300,000	41	17,569	1	429			38	16,283	11	5,994		+	1	429	5	2,142	
	3/25	19,050	3,920,000	49	8,954	2	381	2	381	28	5,905	8	1,524		+	1	191	9	1,715	55
	3/26	24,950	4,170,000	62	15,469	3	749			11	2,745	8	2,745		+			13	3,244	58
	3/27	5,400	4,520,000	56	3,024	9	486	4	216	14	864	15	810		+					60
	3/28	4,400	4,320,000	55	2,420	7	308			14	660	20	968	1	44					52
R 967	3/16	Injected intravenously with 1 mg. T. B.																		
	3/25	5,300	6,150,000	52	2,756	8	424	1	53	4	212	34	1,802					1	53	62
	3/28	Injected subcutaneously and intraperitoneally with 40 cc. trypan blue every 2nd day.																		
	3/29	5,050	5,270,000	77	3,889	7	353			6	303	8	404							52
	3/30	6,150	4,780,000	42	2,583	3	185			42	2,583	9	553	+	+			4	246	54
	4/1	5,400	5,240,000	29	1,566	5	270	1	54	41	2,214	15	810	4	216	5	270			52
	4/3	7,350	5,850,000	24	1,764	11	808			39	2,866	19	1,397		++	3	220	4	294	54
	4/5	5,000	5,060,000	34	1,700	9	450			34	1,700	14	700	5	250			4	200	62
	4/6	5,150	5,110,000	65	3,347	8	412			17	875	10	515							54
	4/8	6,400	5,520,000	51	3,264	6	384	1	64	12	768	17	1,088	7	448	1	64	5	320	56
	4/9	5,800	5,260,000	61	3,538	5	290			13	754	19	1,102	2	116					56
	4/10	18,300	4,820,000	71	14,823	1	183			14	2,562	4	732		++					64
	4/11	5,950	5,260,000	64	3,808	3	178			11	655	14	833	3	179	4	238	1	60	53
	4/12	3,850	5,280,000	40	1,540	6	231			33	1,270	7	270	3	115	5	193	6	231	55
	4/13	12,500	5,020,000	77	9,625	4	500			9	1,125	7	875	1	125	1	125	1	125	52
	4/15	8,950	4,790,000	81	7,339	3	269			9	806	3	269	2	179	1	89			52
	4/16	6,850	4,020,000	77	5,275	5	342			10	685	5	342	1	69	1	69	1	69	44

TABLE I—*Concluded*

Experiment	Date	White blood cells per cu. mm.	Red blood cells per cu. mm.	Neutrophils		Basophils		Eosinophils		Monocytes		Lymphocytes		Giant cells		Clasmatocytes		Unclassified		Hemoglobin per cent
				Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	Per cent	Per cu. mm.	
R 939	5/20	8,450	4,610,000	49	4,140	4	338			23	1,943	22	1,859					2	169	
	5/20	Injected intraperitoneally and subcutaneously with about 40 cc. sterile plain agar containing 10 cc. of India ink.																		
	5/21	26,100	3,690,000	76	19,836	2	522	3	783	14	3,654	5	1,305							
	5/22	18,850	3,810,000	60	11,310					25	4,712	14	2,639		+			1	188	
	5/23	10,300	4,490,000	65	6,695	1	103			27	2,781	7	721							
	5/24	14,850	4,680,000	50	7,425	4	594			23	3,415	19	2,821	3	445			1	148	
	5/27	6,750	4,620,000	47	3,172	3	202			24	1,620	22	1,485	4	270					
	5/29	7,650	4,330,000	67	5,125	2	153			11	841	20	1,530		+					
	5/31	6,500	4,640,000	65	4,225	2	130	3	195	8	520	22	1,430		++					
	6/3	10,100	4,910,000	80	8,080	1	101			2	202	16	1,616		+			1	101	
	6/5	8,750	4,200,000	92	8,050					4	350	3	262					1	87	
	6/6	8,150	4,930,000	86	7,009	8	652			2	163	4	326							

sections. Numerous giant cells were easily seen in the smaller capillaries. Fig. 14 illustrates one of the giant cells in the lung of this animal.

Rabbit R 580 received the same type of injections as R 790.

Trypan blue (1 per cent solution in distilled water) was given intravenously or intraperitoneally in 4 to 10 cc. doses every second day and in addition the animal re-

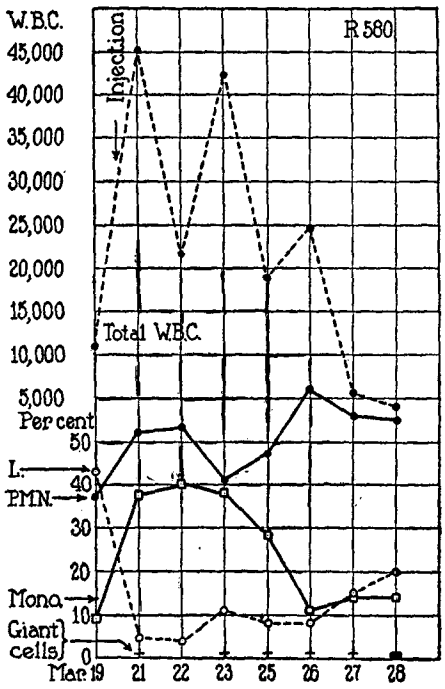

CHART 2. Graph of the white blood cells of Rabbit R 580. The arrow indicates the time at which 40 cc. of sterile plain agar was introduced subcutaneously and intraperitoneally. At this point injections of trypan blue were begun and were continued on every second day.

ceived plain agar into many areas of the subcutaneous tissue and peritoneal cavity. Prior to the injections the cells of the blood were entirely normal (Table I, Chart 2). There were 11,000 leucocytes per cubic millimeter. However, on the day following, the total leucocyte count rose to 45,500 cells per cubic millimeter—an increase of over 400 per cent. This rise was chiefly brought about by the monocytic strain of cells. The neutrophiles also increased somewhat, but the lymphocyte curve descended, their number being represented by only 4 per cent of the total white

blood cells. At the height of the reaction there were over 17,000 monocytes in each cubic millimeter of blood. Many of these monocytes were of the young type previously described. Some of the monocytes and neutrophiles appeared to contain fragments of agar. Many of the neutrophiles had lost a considerable portion of their specific granules. There was a tendency for the leucocytes to be clumped in large masses. Some of the young monocytes contained two oval nuclei.

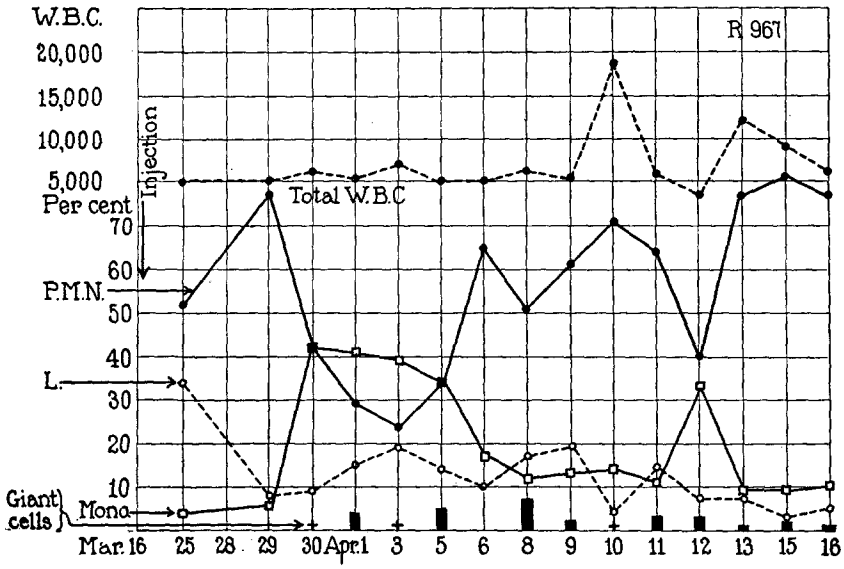

CHART 3. Graph of the white blood cells of Rabbit R 967. The first arrow shows the time at which the rabbit received 1 mg. of avirulent tubercle bacilli intravenously. This presumably did not modify any of the changes found in the chart. The second arrow indicates the time at which 40 cc. of sterile plain agar was administered subcutaneously and intraperitoneally. At this time also injections of trypan blue were begun and they were continued every second day.

2 days after the agar injection, the total leucocyte count was 21,900 per cubic millimeter, but the percentage of monocytes was even higher (40 per cent). The accompanying table and chart (Table I, Chart 2) will make these changes clear. 3 days after the beginning of the experiment the total white blood cell count was again elevated to 42,850 cells per cubic millimeter, but there were no significant changes in the percentages of cells except a rise of lymphocytes from 4 to 11 per cent. Giant cells of both types began to appear in the blood and could be found in practically every smear thereafter. 6 days after the injection, a mild leucopenia set in with the monocytes constituting about 14 per cent of the cells.

Rabbit R 967 was interesting because it showed giant cells in large numbers in the blood stream.

On March 16, 1928, the animal received an intravenous injection of 1 mg. of avirulent tubercle bacilli which did not produce progressive tuberculosis and which presumably caused no significant changes in the blood picture to be described. The blood picture was normal on March 25 when intravenous injections of trypan blue were begun and continued every second day for nine doses. This may account for the presence from time to time of from 3 to 5 per cent of clasmatocytes (macrophages) in the blood. The animal was injected with plain agar in a number of areas on March 28. The chart and table (Table I, Chart 3) show the changes in the total number and percentage values of the cells. The chief result of the injection on the blood was a rather striking and prolonged rise in monocytes together with a maintained decrease in the percentage and absolute number of lymphocytes.

This animal differed from the preceding one in that there was no sharp rise in total leucocytes within a short time after the injection. However, there did occur the marked elevation of percentage of monocytes. This increase was found on the second day and persisted for 1 week during which time the monocytes ranged from 34 to 42 per cent of the circulating white blood cells. During the next 2 weeks monocytes remained abundant, varying between 9 and 33 per cent. Giant cells occurred in the blood 2 days after the agar injection and were found in every film of blood until autopsy 17 days later. They frequently were so numerous as to constitute 2 or 3 per cent of the total number of leucocytes and on one occasion reached as high as 7 per cent. It was not uncommon to find two in a single high power field of the microscope. Most of the "epithelioid giant cells" contained from 2 to 7 nuclei (Fig. 15) but an occasional one had from 10 to 14 nuclei; the foreign body giant cells, on the other hand, contained from 2 to 50 nuclei, many of them having from 15 to 30.

Fig. 2 illustrates what might be called a mononuclear giant cell of the blood. It measured 26.7 by 31.4 microns, about twice the size of an ordinary monocyte. It had a large indented nucleus with a nucleolus, a definite rosette of fine neutral red bodies, together with numberlesss delicate mitochondria. This cell would fall into the monocyte group and would be further qualified by saying that it is a young stimulated form. If the rosette were slightly larger we would call it an epithelioid cell. 4 days after the injection, when monocytes constituted 41 per cent of the white blood cells, this type of cell, the young, large, stimulated form, was very abundant. Cells entirely identical with this except for the presence of 2, 3, or more nuclei were frequently found. Fig. 3 illustrates one of these epithelioid giant cells with 9 nuclei. All intermediate stages between the cells represented by Figs. 2 and 3 could readily be demonstrated in the blood.

At first the mechanism by which the giant cells reached the peripheral veins was not understood for some of these cells were more than

50 microns in their smallest diameters. It is difficult to conceive how these cells could find their way through the capillaries of the lungs and then into the systemic circulation. Figs. 5 and 10 illustrate how this phenomenon is accomplished. The cell in Fig. 10 was taken from the peripheral ear vein of Rabbit R 967 and shows that these giant cells are not rigid but that they may become elongated to such an extent as to pass through very small capillaries. Fig. 5 was from the

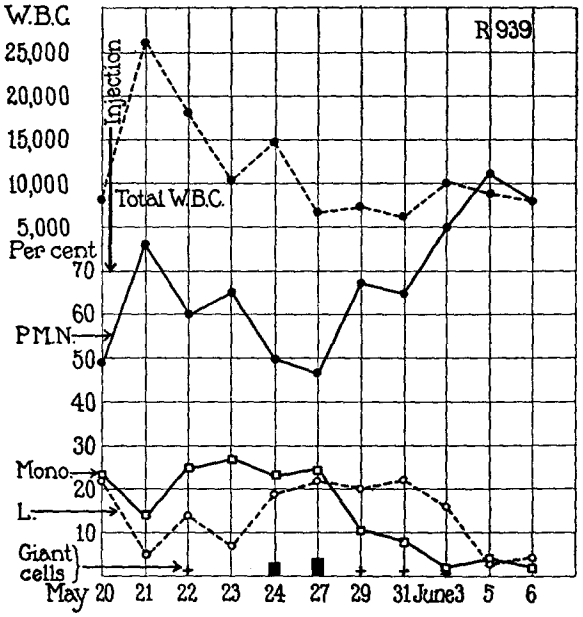

CHART 4. Graph of the white blood cells of Rabbit R 939. The arrow indicates the time at which 40 cc. of sterile plain agar was injected into the subcutaneous tissues and into the peritoneal cavity.

central artery of the ear of Rabbit R 939 and illustrates the apparent ease with which the cells may make their way through the capillary bed.

The next experiment which was tried was to mark the agar in some way in order that it might be identified when it was transported within cells and to distant parts of the body. This was carried out by adding to the 40 cc. of agar to be injected 10 cc. of Higgins' India ink. This was mixed carefully and made an intensely black preparation.

Rabbit R 939 was chosen for the experiment. The count prior to the injection showed the animal to have 23 per cent, an abnormally high value, of monocytes (Table I, Chart 4). On the day following the injection, the total leucocytes rose to 26,100 cells per cubic millimeter. The monocytes rose and remained high for several days. Giant cells of both types appeared in very small numbers in the blood on the second day and were found on several occasions over a period of 2 weeks. Occasionally a few granules of carbon could be seen in the giant cells, but not as much as was expected. Fig. 1 shows an epithelioid giant cell taken from the arterial blood, which has several definite fragments of agar containing carbon. The autopsy on this animal demonstrated that the carbon had not been as evenly distributed in the agar as was expected. The giant cells immediately adjacent to the ink deposits contained it in great abundance, but most held relatively little.

Many more animals were subjected to similar experiments with agar and with other substances. It was not possible to induce regularly an appearance of cells in the blood with any of the substances used except agar. In one animal which had been injected with large amounts of lycopodium spores, one giant cell was found in the blood on a single occasion. This finding could not be repeated.

DISCUSSION

These studies on the peripheral blood have made it possible to form definite ideas about the origin and development of "epithelioid giant cells" and of foreign body giant cells. It becomes necessary to consider these elements histologically as two groups of cells which have a common origin from monocytes. Under the conditions of these experiments it appears that the great majority of foreign body giant cells are formed by the fusion of monocytes, and that the majority of "epithelioid giant cells" are formed by amitotic division of the nucleus or nuclei of epithelioid cells which latter elements are monocytes of large size, with hypertrophied rosettes. The results of the studies would thus confirm the theories of both schools of pathologists, of those who consider that giant cells are the products of fusion, and those who maintain that they form by division of the nuclei. The difference of opinion on this point has resulted doubtless from the failure to recognize that in almost every tissue reaction which produces giant cells, both types are present, but that their relative numbers assume different proportions according to the character of the

substances which excite the response. No evidence has been observed in these studies to support the theory that epithelioid giant cells are formed by the fusion of individual cells. On the other hand, many epithelioid cells and "epithelioid giant cells" with partially divided nuclei, and others which appeared as though the nuclei had recently divided amitotically have been seen.

Regarding the fate of giant cells, no definite conclusions are possible but these studies lend support to the conclusion of Hektoen (28) that foreign body giant cells may separate into their constituent elements. No evidence is available that "epithelioid giant cells" undergo a similar process.

The occurrence of the two types of giant cells in the circulating blood may be explained in either of two ways. They may be formed in the blood vessels in the neighborhood of the foreign body reaction, or they may form extravascularly and migrate into the vessels. The latter explanation would appear more tenable, for giant cells are to some extent motile, and can become so elongated that passage through the capillary walls should be entirely possible. Furthermore, it would be difficult to explain the presence of large pieces of agar in the cells, as shown in Figs. 4 and 9, by any other hypothesis than that they have been carried within cells from the tissues into the blood.

SUMMARY AND CONCLUSIONS

1. It has been demonstrated that giant cells of the foreign body and epithelioid types can be induced to appear in the blood stream.

2. Evidence has been presented which indicates that foreign body giant cells are primarily formed by fusion of monocytes and that the fate of these giant cells is accomplished, at least in some instances, by a separation into the constituent elements.

3. Further evidence has been presented which lends support to the hypothesis that "epithelioid giant cells" reach their stage of evolution, not by fusion of monocytes, but by amitotic division of the nuclei of monocytes and epithelioid cells.

4. The presence of giant cells in the peripheral blood as the result of agar injections is almost invariably associated with, or preceded by a marked monocytosis in which the new monocytes are of large size and show evidence of immaturity.

5. Injections of agar into the tissues result in decreased absolute and percentage values of lymphocytes and a diminution of the specific granules in many of the polymorphonuclear leucocytes.

6. It would appear from these studies that a clear differentiation of "epithelioid giant cells" and "foreign body giant cells" in the blood is usually possible, but that on the other hand, a few cells may be present which have some of the characteristics of each type. These latter cells probably represent in their formation both a fusion of individual cells and an amitotic division of the nuclei of monocytes.

7. Clasmatocytes or macrophages have in rare instances been seen to take part in the formation of foreign body giant cells. At least one instance has been noted of the fusion of a clasmatocyte with several monocytes. No evidence is available to demonstrate that macrophages ever play a part in the formation of "epithelioid giant cells."

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EXPLANATION OF PLATES

PLATE 10

Drawings of cells from arterial and venous blood of rabbits after injections of agar; stained with neutral red and Janus green and drawn while still alive.

FIG. 1. An epithelioid giant cell from the peripheral arterial blood of Rabbit R 939. Small aggregations of carbon can be seen. The mitochondria are very abundant and are best seen at the periphery of the rosette. This cell measured 39.3 x 33 microns.

FIG. 2. A huge, young, stimulated monocyte from Rabbit R 967. It is almost as large as the cell in Fig. 1, but contains only a single nucleus with a large nucleolus. Thousands of delicate mitochondria are stained with Janus green and a typical dense rosette is stained with neutral red. Cells identical with this, except for the presence of two or more nuclei, could be found in practically every field. This cell measured 31.4 x 26.7 microns.

FIG. 3. Epithelioid giant cell from venous blood of Rabbit R 967, with nine nuclei. It has a unified central rosette, similar to those of Figs. 1 and 2. Size 36.1 x 33 microns.

FIG. 4. Three monocytes which have fused around a piece of agar in the blood of Rabbit R 790. This animal had received trypan blue intravenously on several occasions and the agar foci were stained in an identical manner, as illustrated in this figure. When first seen this cell was practically spherical, but after 2 hours the three constituent cells were almost entirely independent of each other. Size 35 microns at widest diameter.

FIG. 5. This huge giant cell was obtained from a peripheral artery of Rabbit R 939. It measured 107 microns in length. It illustrates very well that these cells are capable of changes in shape so great as to enable them to pass through the capillaries. A red blood corpuscle is shown to give an idea of their great sizes.

FIG. 6. A foreign body giant cell with many nuclei from the venous blood of Rabbit R 579. This cell and the monocyte nearby are filled with masses of irregular, fragmented material. This material is probably agar undergoing changes within the cell. Size 67.5 × 47.1 microns.

FIG. 7. A trinucleated cell containing the same general type of material as that in the cell of Fig. 6. The mitochondria have faded somewhat. This cell is also from the blood of Rabbit R 579. Size 21 microns in diameter.

FIG. 8. Another foreign body giant cell from the blood of the same animal. Size 28 × 17 microns.

FIG. 9. A foreign giant cell from the blood of Rabbit R 790. There is a large, lobulated mass of material in the center of the cell which is probably agar that has not as yet undergone very much change within the cell. In the upper left region of the cell two rosettes can be seen. Size 84 x 52.5 microns.

FIG. 10. Another elongated cell. This foreign body giant cell was from the blood of Rabbit R 967. Size 73.8 by 14 microns.

PLATE 11

Four photographs of living cells supravitally stained with neutral red and Janus green, and one photograph (Fig. 14) from a section.

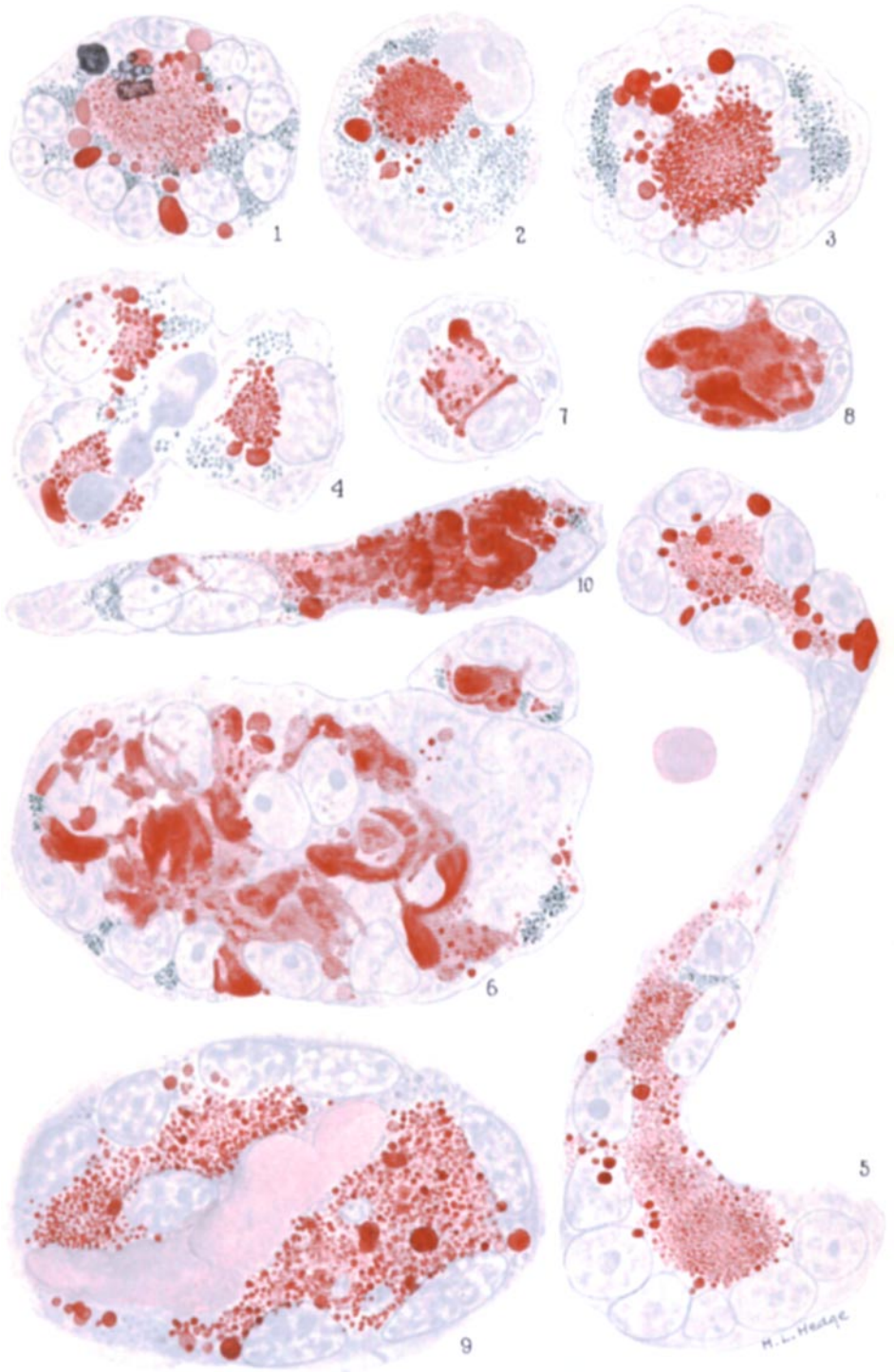
FIG. 11. Photograph of a large foreign body giant cell from the blood of Rabbit R 790. It has very many nuclei scattered throughout the cell and the cytoplasmic bodies are arranged in no definite pattern. Several monocytes can be seen on the surface of the cell, as if in process of fusion. Red blood corpuscles can be seen for comparison of size. Magnification × 575. Stained with neutral red in supravital preparation.

FIG. 12. Photograph of a foreign body giant cell in the blood with many monocytes and epithelioid cells about it. This animal, R 939, had received agar plus India ink which was injected under the skin and into the peritoneal cavity. Note the definite rosettes of neutral red bodies in the monocytes and epithelioid cells. Magnification × 750. Stained with neutral red in supravital film.

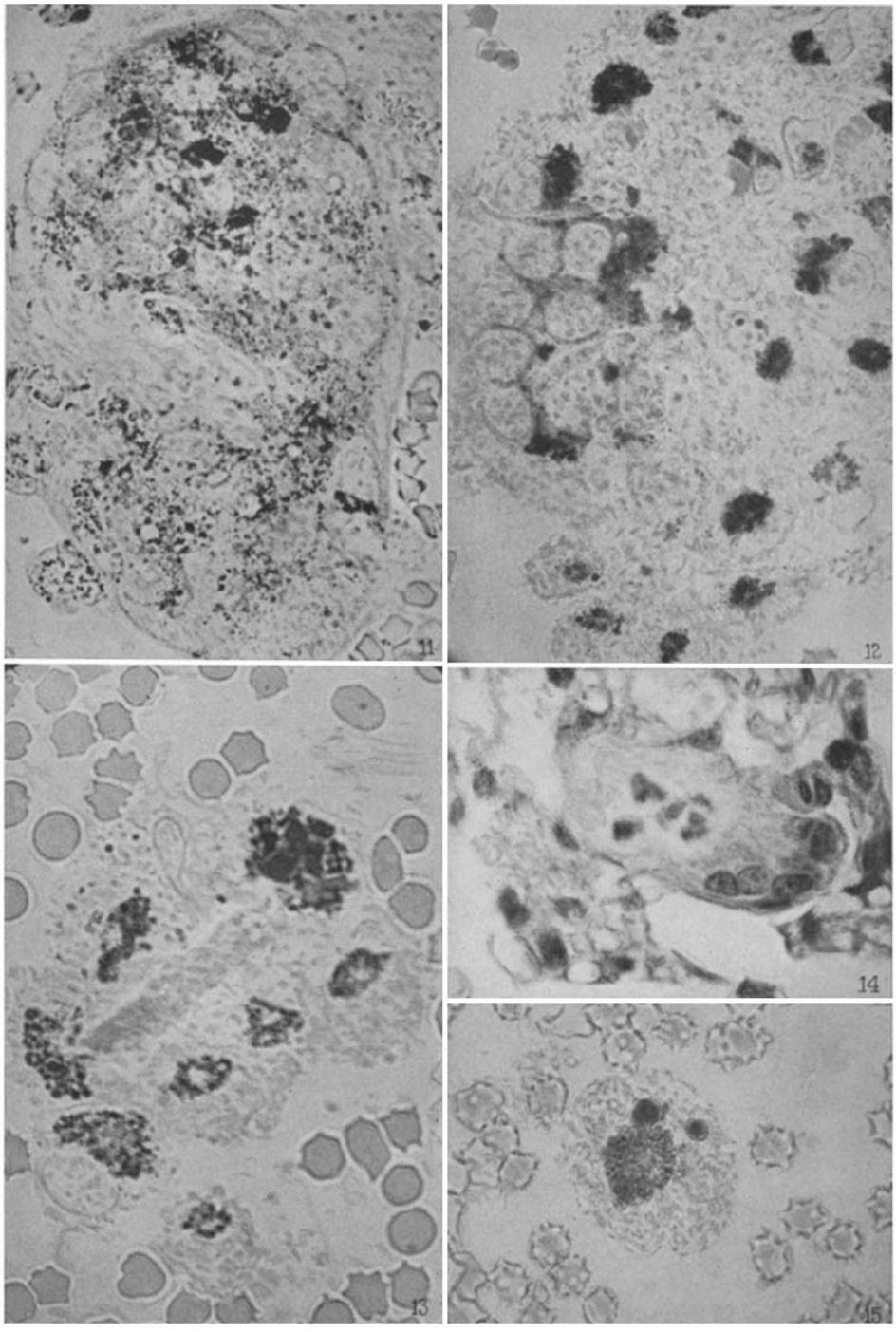
FIG. 13. Photograph of an accumulation of monocytes about a foreign body in the blood of Rabbit R 967. The cell at the upper end of the figure is a clasmatocyte. The cytoplasm of these cells could not be separated. They were apparently fused. A single monocyte may be seen nearby at the lower border of the figure. Magnification × 750. Stained with neutral red by means of the supravital method.

FIG. 14. Photograph of a giant cell in a capillary of the lung of Rabbit R 579. Many giant cells could be demonstrated in the lungs of this and other animals similarly treated. Magnification × 750. Stained in fixed section with hemotoxylin and eosin.

FIG. 15. Photograph of an epithelioid giant cell in the blood of Rabbit R 967. Note the unified central rosette and peripheral nuclei. Contrast with Fig. 11. Magnification × 750. Stained with neutral red.



(Forkner: Multinucleated giant cells in blood)



(Forkner: Multinucleated giant cells in blood)