CONCERNING VESICULOCYTOSIS

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During the preparation of specimens from blood and tissues for detection of autoantibody-producing plaque-forming cells, if hypertonic (10-15%) salt solutions are used instead of physiological saline a phenomenon of false plaque formation takes place through the liberation of the cell contents into the surrounding medium and pycnosis of the cells. This phenomenon takes place with the blood karyocytes of animals of different species and of man when various salts are used, such as sodium nitrate, ammonium sulfate, calcium chloride, etc. False plaque formation in hypertonic solutions does not take place with all cells in the preparation, i.e., it depends on their state. The phenomenon is particularly strongly expressed in the period of maximal intensity of the cellular immune response.

KEY WORDS: *plaque-forming cells.*

The term "vesiculocytosis" was suggested by Kislyakov [1] to describe the phenomenon of formation of an erythrocyte-free zone around a nucleated cell, which is very similar to the formation of a plaque of local hemolysis by Jerne's method. Kislyakov rightly considers that the formation of this zone (mainly around polymorphs) is the result of separation of the erythrocytes by the contents of a nucleated cell, liberated from it into the surrounding medium.

Since Kislyakov used my modification [2] of Jerne's method to detect autoantibody-producing cells as the basis of his technique, in this investigation I set out to discover the nature of the phenomenon observed by him and also the possibility of using it for immunological investigations.

EXPERIMENTAL METHOD AND RESULTS

The formation of a plaque of hemolysis in Jerne's method is connected with the vital activity of the cell, for which definite conditions (time, temperature, composition of the medium, and so on) are created, of which the most important is the presence of isotonicity of the solution in which the cells are kept. This condition, in Kislyakov's paper cited above [1], is not satisfied, for he mixes fresh blood with 10 volumes of hypertonic (10%) sodium citrate solution. As a result extraction of the cell contents may take place, forming a "cloud" around the cells, which may also separate the erythrocytes.

To prove this statement, I studied the action of hypertonic (15%) solutions of various salts, added to 1 drop (0.02 ml) of citrated blood diluted 1:3 on a slide. The final concentration of the salt was thus only half that used in Kislyakov's method (5%). At the same time a preparation was made from the same blood with the addition of 0.01 ml physiological saline, as in my method. The drops were covered by coverslips, the edges of which were smeared with Vaseline 1-2 mm from the side facing the preparation. After being kept for 1 h at room temperature the preparations were kept overnight on the bottom shelf of a refrigerator, after which the number of plaques of hemolysis was counted. The results obtained by the study of the effect of hypertonic solutions of various salts on karyocytes of animal and human blood are given in Table 1.

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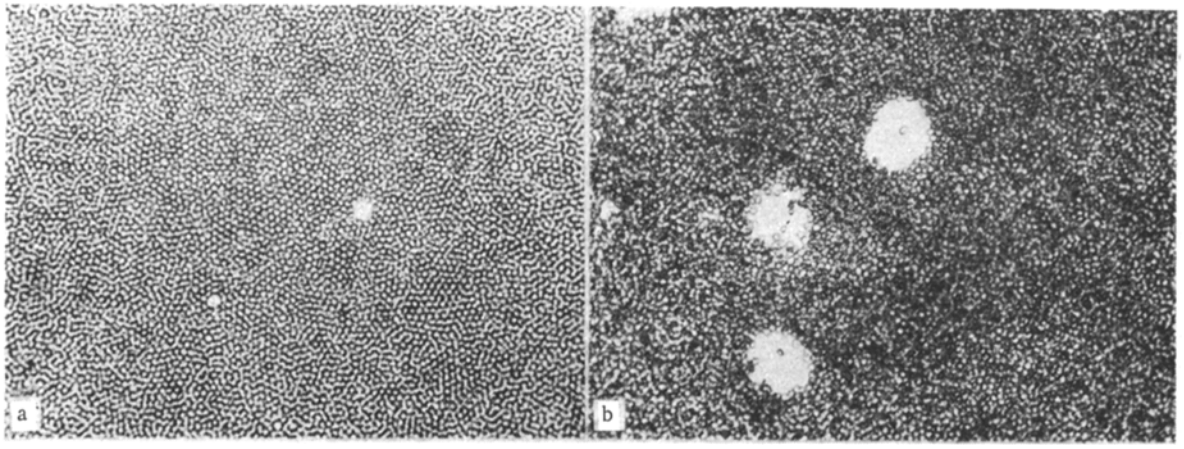

Fig. 1. Photomicrograph of blood cells forming autohemolysins (a) and false plaques in 15% hypertonic sodium nitrate solution (b) (healthy mice); 200×.

TABLE 1. Effect of Addition of 15% Solutions of Electrolytes and Physiological Saline in a Volume of 0.01 ml to Blood Preparations Made by Jerne's Method in Klemparskaya's Modification

Leukocytes tested*	Mean number of karyocytes on addition of acetic acid	Mean number of plaques in field of vision (in %) after addition of various solutions										
		physiological saline	magnesium sulfate	potassium chloride	sodium sulfate	sodium nitrate	barium chloride	ammonium chloride	ammonium sulfate	calcium chloride	sodium chloride	sodium citrate
Human	66±4	0,1±0,01	0,5±0,1	5±0,2	4±0,1	12±2†	Agglutination of erythrocytes	2±0,5	6±1	15±2,5†	18±1†	2±0,1
Monkey	75±8	2±0,1	2±0,5	2±0,3†	3±0,2	22±2†	8±1	8±1	1±0,2	3±0,3	11±2	3±1
Guinea pig	150±5	1±0,1	2±0,4	2±0,5	3±0,1	14±2†	8±1	0,1±0,05	3±0,2	5±2	5±0,1†	2±0,1
Dog	35±5	0,5±0,1	0	0	0	2±0,3†	Agglutination of erythrocytes	Agglutination of erythrocytes	2±0,2	1±0,3	8±1†	Agglutination of erythrocytes
Albino mouse (noninbred)	57±3	1±0,3	4±0,2	3±0,1	2±0,1	23±0,2†	6±0,2	8±0,5†	2±0,2	11±1,3†	3±0,1†	2±0,3
Sheep	42±4	0,4±,01	8±2	13±3†	8±3	7±1†	9±2†	3±0,1	6±1†	3±0,5	7±1	3±0,4
Rat	64±6	0	0	0	0	22±4†	0	0	0	0	3±0,6	2±0,4

*0.03 ml 5% sodium citrate and 0.13 ml physiological saline added to 0.1 ml blood; mixture diluted 1:2 with physiological saline.

†Preparations with considerable increase in size of plaques.

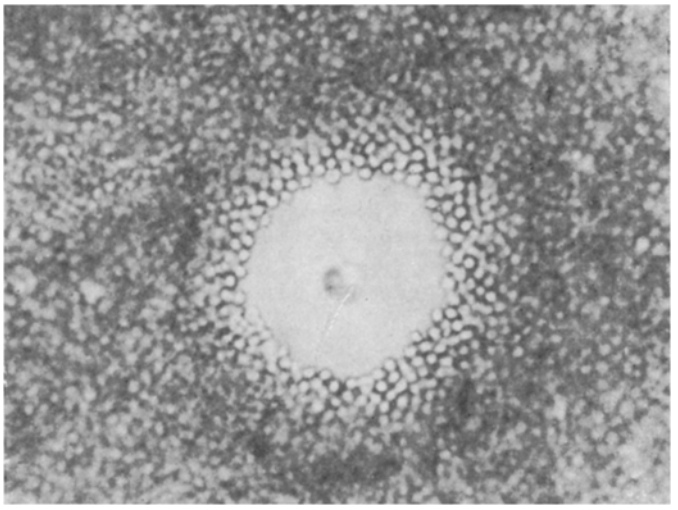

Fig. 2. Plaque of local autoimmune hemolysis in preparation of mixture of spleen cells with autologous blood taken from mouse 24 h after injection of 50 mg bovine albumin.

Addition of hypertonic solutions of various salts in fact caused the formation of zones around the karyocytes very similar to plaques of hemolysis (Fig. 1), and not only the number of zones, but also their size was increased. This phenomenon was particularly marked in the case of addition of sodium nitrate and of calcium, potassium, and sodium chlorides. Some salts (lithium sulfate, potassium dihydrogen phosphate, barium chloride, etc.) caused coagulation and agglutination of the erythrocytes, and no distinct zones could be seen. Solutions of copper sulfate caused the formation of very large zones around the karyocytes, with rims of clouded erythrocytes at their edge. These formations differed sharply from ordinary plaques. It is interesting to note that well-marked species differences were found in the response of the karyocytes to an increase in osmotic pressure of the surrounding medium. This phenomenon was most clearly demonstrated in preparations of mouse and sheep's blood, less clearly in preparations of rat blood. As Table 1 shows, even at maximal intensity, false plaques were not formed by all cells, i.e., the process depends on the species, age, and functional state of the cells.

The sensitivity of the karyocytes to an increase in osmotic pressure of the medium was particularly strongly increased in the period of maximal manifestation of cellular immune responses: for example, on the fourth day after intraperitoneal injection of 0.5 ml of a 20% suspension of sheep's erythrocytes into mice. Before immunization the percentage of cells forming autoantibodies in the spleen was 0.11 ± 0.064 with physiological saline, 10.5 ± 1.76 with the addition of 10% sodium citrate solution, and 16.1 ± 1.36 with the addition of 20% sodium chloride solution. On the fourth day, 14.5 ± 1.63, 20.1 ± 2.86, and 62.4 ± 11.43%, respectively, were obtained.

Plaque formation following the action of hypertonic solutions on the cells, in the writer's opinion, takes place as the result of extraction of the contents from them to form a gelatinous "cloud" around the remnants of the nucleus and cytoplasm.

After incubation in hypertonic solutions of sodium chloride or citrate for 1 h at 37°C the suspension of mouse spleen cells becomes gelatinous in consistency and its liquid part can be obtained for the determination of its optical density only after addition of an equal volume of physiological saline, pressing through a double layer of gauze, and centrifuging for 10 min at 3000g. Compared with the optical density (measured by the FÉKM-56 instrument with a No. 6 filter) of the suspension fluid in samples with isotonic physiological saline (0.028 ± 0.009), even in this diluted fluid of the samples with hypertonic medium it was more than five times greater (0.159 ± 0.04 and 0.145 ± 0.03).

The fact that the cells contract under these circumstances is confirmed by the decrease in their size in preparations stained by the May–Gruenwald method: from 5-7 μ in preparations using physiological saline to 1-2 μ in films from a suspension in hypertonic medium.

It will thus be clear from the facts described above that the formation of plaque-like zones around karyocytes in hypertonic solutions is an artifact and that this method cannot be used in immunological investigations. Evidence additional to that already published [3, 4] of the immunological nature of the plaques observed in preparations made by my original (un-

changed) modification of Jerne's method is given by reproduction of Andersen's phenomenon of immune blocking [5] and the recording of translucency and lysis of erythrocytes in the peripheral zone of the plaque (Fig. 2).

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ANTIGEN-DEPENDENT INDUCTION OF A NONSPECIFIC HUMORAL FACTOR BLOCKING ROSETTE-FORMING CELLS in vitro AND in vivo

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Isologous serum of mice immunized with rabbit globulin (IARS) was shown to contain a factor inactivating rosette-forming cells (RFC) in vitro from CBA mice immunized with sheep's erythrocytes. If the mice were immunized with sheep's erythrocytes after preliminary injection of IARS, the number of RFC at the peak of the immune response was about 30% of their number in mice receiving normal isologous serum together with sheep's erythrocytes. The decrease in the number of RFC took place on account of cells not containing θ antigen. Passive immunization with IARS did not affect proliferation of antibody-forming cells or synthesis of antibodies against sheep's erythrocytes.

KEY WORDS: *rosette-forming cells; antibody-forming cells; θ antigen; isologous antirabbit serum.*

The possibility of uncoupling the processes of antibody production and accumulation of rosette-forming cells (RFC) during immunization of mice with sheep's erythrocytes (SE) was demonstrated previously: This result was obtained by preliminary injection of foreign protein into the same animals [2].

It was decided to investigated the mechanism of the above phenomenon since it provides an approach to the explanation of processes regulating the kinetics of RFC in the course of the immune response.

EXPERIMENTAL METHOD

SE and rabbit immunoglobulins, obtained by precipitation with ammonium sulfate at 40% saturation, were used as the antigens. The immunoglobulin preparations were purified from large aggregates by ultracentrifugation of 105,000g for 2 h at 20°C. The immunoglobulin preparations did not contain hemagglutinins against SE.

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