ANTIGENIC ANALYSIS OF NUTRIENT MEDIA USED FOR IN VITRO CELL CULTURES

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Recent investigations have been concerned with the immunological characteristics of cells [3, 4, 6], including cells cultivated in vitro [10, 12]. The immunogenetic aspect of these investigations is of greatest interest [14, 15, 17]. The loss of some antigens has been observed in the process of cultivation [16, 18]. Other antigens, however, are preserved even after prolonged cultivation [2, 7, 8]. The secretion of γ-globulin into the medium by HeLa cells has been reported [11].

The object of this investigation was to study the difference between a medium before use for cultivation of cells (medium A) and the medium after cultivation of cells (medium B) by immunological methods, i.e., to detect a specific antigen in the medium after its use for cultivation of cells, to investigate the fate of the protein component, and to compare the media with various sera.

METHOD

Cells were cultivated in synthetic nutrient medium No. 199 with 10% serum. As serum component the serum from a young calf and the serum from adult chinchilla rabbits were used. The seeding dose was 500,000 cells to 10 ml of medium. The cultivation time was 7 days, with one change of medium. After cultivation of the cells the media were collected for immunological analysis.

The following strains of cells were used: HeLa – a line of cells from a carcinoma of the human uterus [13], A-1 – a line of cells from human amnion (obtained in 1959 by T. G. Orlova at the Moscow Research Institute of Viral Preparations), CK – a line of cells from the lung of a human embryo [9], Cave – a line of cells from carcinoma of the stomach [1], Cave K_1 – a clonal line of cells from a carcinoma of the human stomach, Cave K_r – a clonal line of cells from a carcinoma of the human stomach adapted to rabbit serum.

The anaphylaxis with desensitization reaction was carried out on guinea pigs weighing 250-300 g. The guinea pigs were sensitized by subcutaneous injection of 0.5 ml of medium B of line HeLa. The desensitizing and reacting injections of the antigens were given into the heart 3 weeks after the primary injection of protein.

The following antisera were used: antiserum to medium A, antiserum to medium B of line Cave, antiserum to medium B of Cave K_r, antiserum to calf serum, and antiserum precipitating human protein. The antisera to media A and B Cave were additionally concentrated.

Scheme of immunization. The antigens were injected intravenously three times on alternate days, and 3 weeks later the animals were reimmunized intraperitoneally. The dose of antigen for immunization, calculated in relation to the serum component, was 1 ml serum, and the dose for reimmunization was 2 ml serum. The protein content in the antigens of media A and B was also determined as nitrogen by Conway's method. The total protein content of medium A was 390 mg, and of medium B 360 mg. Blood was taken twice at an interval of 1 week. The first time of taking blood was 1 week after the last injection of antigen.

The following test antigens were used: calf serum, rabbit serum, human serum of blood group IV, medium A, medium B of line A-1, CK, Cave, Cave K_1, Cave K_r.

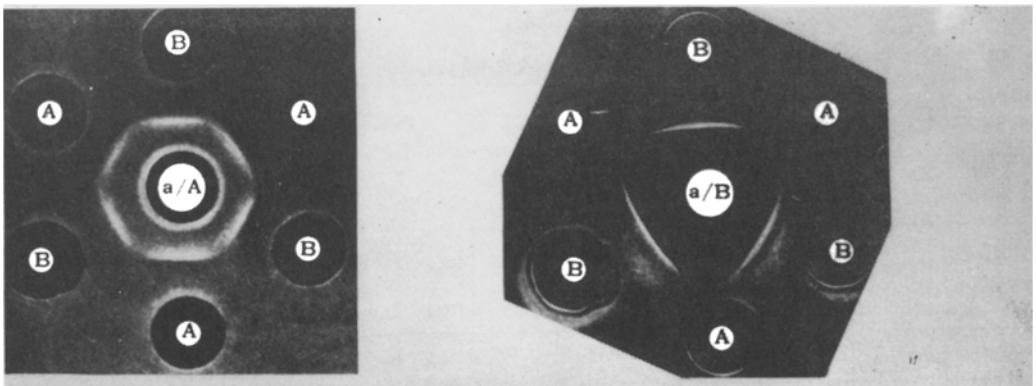

Fig. 1

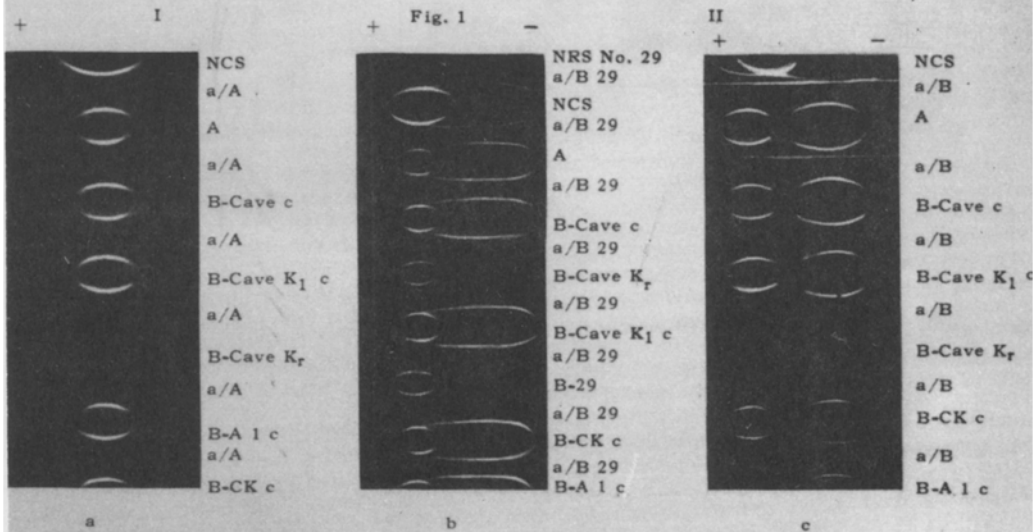

Fig. 2

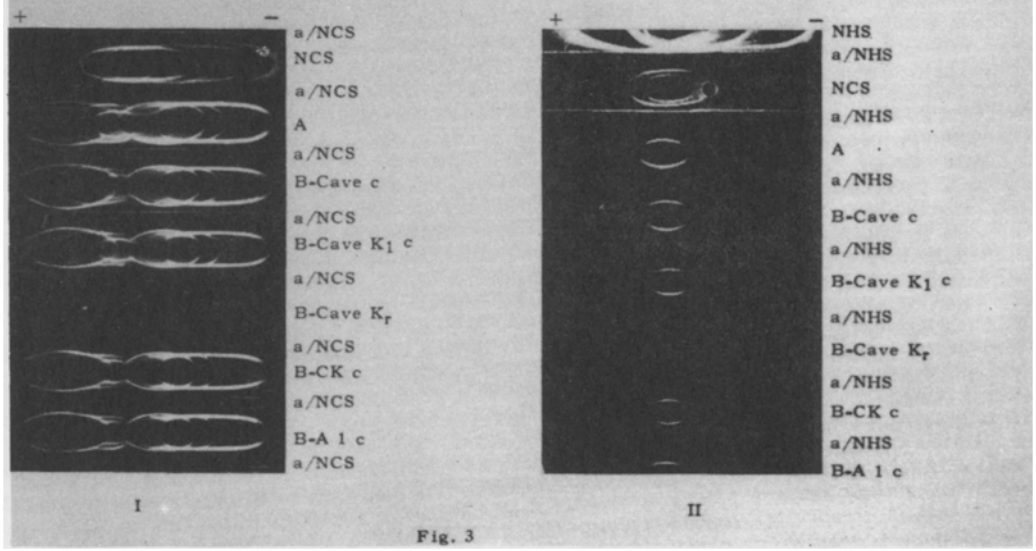

Fig. 3

Fig. 1. Precipitation of antisera to media A (I) and B (II) in gel.

Fig. 2. Immunoelectrophoresis of antisera to media A (a), B-29 (b), and B (c).

Fig. 3. Immunoelectrophoresis of sera precipitating calf (I) and human (II) protein. Conventional signs in all figures. Antisera: a/A) to medium A; a/B) to medium B; a/B 29) to medium B of line Cave K_r; a/NCS) precipitating calf protein; a/NHS) precipitating human protein. Antigens: A) medium before cultivation of cells; B) medium after cultivation of cells; Cave, CK, A-1) names of lines of cells; K_1) clonal line of cells; c) medium with calf serum; K_r) medium with rabbit serum; 29) medium with serum of rabbit No. 29, NCS, NRS, and NHS) normal calf, rabbit, and human sera respectively.

TABLE 1. Results of Antigenic Analysis of Nutrient Media after Cultivation of Cells of Strain HeLa

Guinea pig No.	Sensitization with medium B	Desensitization with medium A										Reacting injection of medium B			
	Dose (in ml)	Dose (in ml)	Reaction	Dose (in ml)	Reaction	Dose (in ml)	Reaction	Dose (in ml)	Reaction	Dose (in ml)	Reaction	Dose (in ml)	Reaction	Dose (in ml)	Reaction
	s/c	i/p		i/c		i/c		i/c		i/c		i/c		i/c	
1	0.5	1.0	+++	1.0	++++	–	–	–	–	–	–	–		–	
2	0.5	1.0	+++	0.25	+++	1.0	++	1.0	–	1.0	–	1.0		++	
3	0.5	1.0	+++	0.25	+++	1.0	++	1.0	–	1.0	–	1.0		++	

Legend: – no reaction; + brief scratching of nose with paw; ++ stronger scratching, sneezing, panting, untidiness of the hair, cough; +++ the same, but more marked, passage of urine and feces; ++++ convulsions, jumping, fits, usually terminating in death of the animal; s/c) subcutaneous injection; i/p) intraperitoneal injection; i/c) intracardiac injection.

For the immunoelectrophoretic analysis the antigens were dispersed on the glass of a photographic plate measuring 9 × 12 cm. A layer of agar with a volume of 10 ml was applied to the plate. The thickness of the layer was approximately 2 mm. The agar was prepared in veronal-medinal buffer at pH 8.6 and with an ionic strength of 0.025. The apparatus for immunoelectrophoresis was slightly modified from that normally used: the paper bridge between the plate and the buffer was replaced by one made of agar. The gel-diffusion reaction was carried out by Ouchterlony's method in Petri dishes of Orion type [5].

RESULTS

The medium B of line HeLa was tested for the presence of specific antigen by means of the anaphylaxis with desensitization reaction (Table 1).

The results showed that, after complete desensitization with medium A and verification of the completeness of desensitization, the guinea pigs reacted by anaphylactic shock to injection of medium B of line HeLa. This indicated that medium B contained a specific protein, resulting from the metabolism of the cells in vitro.

After antisera had been given to media A and B of line Cave, it was possible to investigate the antigenic properties of the nutrient media by gel-diffusion and immunoelectrophoretic techniques. In crossed gel-diffusion tests, antiserum to medium A revealed one antigen in medium A and one antigen in medium B of line Cave (Fig. 1I); antiserum to medium B of line Cave revealed 1 and 2-3 antigens respectively. In the non-identity reaction, medium B of line Cave contained an additional non-identical antigen (Fig. 1II). The results demonstrated an antigenic difference between medium A and medium B of line Cave, namely that medium B was richer in antigens than medium A.

In the immunoelectrophoretic tests the antisera were studied in more detail. Additional test antigens were used: calf serum, medium B of line Cave K_l, Cave K_r, CK, and A-1. Calf serum was used for identification of serum antigens, medium B of line Cave K_l for comparison of the original line with the clonal line, medium B of line Cave K_r for detection of the specific antigen bound with cells of line Cave but not containing calf serum, and medium B of lines CK and A-1 for determination of specificity.

In the course of immunoelectrophoresis antiserum to medium A revealed the presence of one antigen in media A and B of line Cave, which was also found in medium B of line Cave K_l, CK, and A-1. These antigens were not found in medium B of line Cave K_r. Two antigens were detected in calf serum (Fig. 2a). Antiserum to medium B of line Cave revealed the presence of 3-4 antigens in medium B of line Cave and in medium A; the same number of antigens was also found in medium B of lines Cave K_l, CK, and A-1. In medium B of line Cave K_r no antigens were found. The calf serum contained 6 antigens (see Fig. 2c). These investigations showed that the antigens detected by the antisera were evidently of calf origin and were associated with the presence of calf serum in the

TABLE 2. Results of Immunoelectrophoretic Analysis of Nutrient Media Used for Cultivation of Cells in Vitro

Test antigen	Antisera					Concentrated antisera	
	To medium A	To medium B of line Cave	To calf serum	To human serum	To medium B of line Cave K_r	To medium A	To medium B of line Cave
Human serum	–	–	–	16	–	–	–
Calf serum	–	–	12	3	3	2	6
Medium A	1	1	9	2	3	1	3-4
Medium B of line Cave	1	2	9	2	–	1	3-4
Medium B of line Cave K_1	1	–	9	2	2	1	3-4
Medium B of line Cave K_r	0	–	0	0	1	0	0
Medium B of line CK	1	–	9	2	2	1	3-4
Medium B of line A-1	1	–	9	2	2	1	3-4

Legend: numbers indicate number of precipitation bands; – no test carried out.

nutrient medium. Further evidence of this was the absence of these antigens from medium B of line Cave K_r. In medium B of line Cave K_r, however, no specific antigen resulting from the metabolism of the cells in vitro could be discovered by means of this reaction.

The fate of the serum component of the media was studied by means of a strong antiserum to calf serum. The reaction was carried out in a similar manner to that used in the study of the experimental antisera. In the electrophoretic test this antiserum revealed the presence of 12 antigens in the calf serum and 9 antigens in medium A; however, 9 antigens also were found in each of the media B of lines Cave, Cave K_1, CK, and A-1. Not one antigen was found in medium B of strain Cave K_r (Fig. 3I). The results of this investigation show that the serum component of the medium remained unchanged in the course of cultivation.

The use of an antiserum precipitating human protein was especially interesting. The reaction was carried out in the same way as during the study of the experimental antisera. To determine the properties of the antiserum, in addition normal human serum was used. In the immunoelectrophoretic test this antiserum revealed the presence of 16 antigens in human serum, 3 antigens in calf serum, and 2 antigens each in medium A and medium B of lines Cave, Cave K_1, CK, and A-1. In medium B of strain Cave K_r not one antigen was found (Fig. 3II). In this reaction heterogenic antigens of calf origin were demonstrated.

The use of an antiserum which would not react with the serum components of the media was also of great interest. For this purpose rabbits were immunized with medium B containing rabbit serum. In the immunoelectrophoretic test this antiserum (to medium B of line Cave K_r, adapted to rabbit serum) revealed 1 antigen in medium B of line Cave K_r, no antigen in rabbit serum, 3 antigens in medium A, 2 antigens in medium B of lines Cave, Cave K_1, CK, and A-1, and 3 antigens in calf serum. The position of the precipitation band with its own antigen did not coincide with that of the precipitation bands found in other antigens (see Fig. 2b), thus indicating their specificity. The result of the immunoelectrophoretic tests are given in Table 2.

The comparative study of the medium before and after cultivation of the cells by means of the anaphylaxis with desensitization reaction revealed the presence of a specific antigen in the medium after cultivation of the HeLa cells. The investigation of the medium after cultivation of cells adapted to rabbit serum yielded some interesting results. No antigens were found which were common to this medium and the medium containing calf serum; likewise no common antigen could be found in the medium after cultivation of the cells of this line in calf serum. The strong antiserum to calf serum did not reveal a single common antigen. A strong antiserum precipitating human protein likewise failed to reveal an antigen, although it readily detected a heterogenic antigen with calf serum. In the process of adaptation the antigenic properties associated with the calf serum component of the medium were evidently completely lost.

The antiserum against the medium after cultivation of the cells adapted to rabbit serum evidently revealed a specific antigen, although this same antiserum also revealed heterogenic calf serum antigens in media with calf serum which were not found in the original antigen, confirming that the calf serum antigen was lost in the process of adaptation of the cell lines to rabbit serum. Antiserum to calf serum, i.e., the serum usually added to synthetic nutrient medium No. 199, revealed a large number of antigens both in its own antigen and also in a 10% solution with medium No. 199. However, during cultivation of the cells of all the lines investigated, not one of the antigens of calf serum was lost. Evidently the cells either did not utilize the serum component of the nutrient media, or they did so in a negligible amount and the difference was too small for detection by immunoelectrophoresis.

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