AUTOSENSITIZATION REACTION IN VITRO

CONTACTUAL AGGLUTINATION OF SENSITIZED LYMPH NODE CELLS IN BRAIN TISSUE CULTURE ACCOMPANIED BY DESTRUCTION OF GLIAL ELEMENTS*

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Although passive transfer of allergic encephalitis in rats has been successfully demonstrated by Paterson (1) and ourselves (2, 3), problems inherent in this *in vivo* system made it difficult, if not impossible, to predict the outcome of any given experiments of this series. The study of this phenomenon was thus relegated to the realm of experimental curiosity with no possibility of extending our observation to the more intricate aspects of the condition. With this in mind, a search was made for a method which would enable a more thorough investigation of the immunological reaction of the host to the brain tissue antigen. Since the tissue culture system appeared to be a promising tool in pursuing this study, experiments on the reaction between sensitized lymph node cells and brain cells maintained in tissue culture (4) were undertaken. Successful results of these observations are reported herein.

Materials and Methods

Tissue Culture.—Brain tissue obtained from newborn puppies was cut into small fragments and washed several times in phosphate buffered saline (PBS) containing 3 per cent inactivated bovine serum. The tissue fragments were transferred to an Erlenmeyer flask containing trypsin (0.25 per cent Difco trypsin in PBS with 3 per cent of bovine serum). The flask was then placed on a magnetic mixer and the fragments were stirred at a slow rate for a period of 20 minutes. After removal from the mixer, the flask was allowed to stand for about 1 minute before the supernatant fluid was removed and discarded. More trypsin was added, the same procedure was repeated and the supernatant again discarded. The fluid from subsequent trypsinization procedures was collected in 40 ml centrifuge tubes and placed in the refrigerator until the entire process was completed. The cells were then centrifuged at 800 RPM for 10 minutes in a refrigerated centrifuge. The trypsin solution was decanted and discarded. The cells were washed in Eagle's medium (5), centrifuged again for 10 minutes at 800 RPM, and finally resuspended in Eagle's medium. The cells were counted with a hemocytometer and dispersed in Petri dishes containing two coverslips. The cultures were incubated at 37°C in 5 per cent CO_2 incubator and the medium changed 48 hours later.

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The nutrient medium consisted of Eagle's medium with double concentration of vitamins and amino acids. Calf serum inactivated for 30 minutes at 56°C was added to the medium to a final concentration of 10 per cent.

Animals.—Female rats, 8 to 10 weeks old, of homozygous Lewis strain were injected intradermally with guinea pig spinal cord suspension in complete Freund's adjuvants prepared according to a previously described method (2). The animals were sacrificed by exsanguination 6 to 8 days after injection of antigen unless stated otherwise, and the draining axillary, brachial, and inguinal lymph nodes removed aseptically. The control animals which received injections of Freund's adjuvants *without* spinal cord tissue were sacrificed at the same time. On occasion, lymph node tissue was also obtained from normal Lewis rats. Blood from the sacrificed animals was allowed to clot, and the serum was used for absorption tests (see below).

Lymph Node Cells.—Lymph nodes were teased apart and a dispersed suspension of cells was obtained by passing the contents of the lymph node through a 20 mesh sieve according to the method described by Billingham and Silvers (6). After centrifugation and washing, the number of viable cells was determined by counting cells stained with trypan blue. The concentration of the lymph node cells was then adjusted to the number desired and the material used immediately in the tissue culture system.

Cells obtained from rats injected with guinea pig cord tissue and adjuvants will be referred to as "sensitized" cells, and those obtained from lymph nodes of rats which received the adjuvant mixture alone as "aggressive" cells.

Sera.—Sera were obtained from animals of the following experimental groups:

(*a*) Normal rats
(*b*) Rats injected with guinea pig cord and adjuvants 7 to 10 days before
(*c*) Rats injected with guinea pig cord and adjuvants and recovered from paralysis
(*d*) Rats injected with adjuvants alone and sacrificed at the same interval after inoculation as group *b*.

Serum was used either when freshly drawn or after storage at subfreezing temperatures.

Serum Absorption.—A pellet of packed lymph node cells obtained by centrifugation of the suspension at 1200 RPM for 10 minutes was mixed in equal amounts with rat serum obtained from the four experimental groups mentioned above.

The mixtures were incubated at 37°C for 45 minutes with frequent agitation. The serum was then removed and the cells washed once in PBS-saline solution. They were then counted to obtain the desired concentration for contact reaction with the tissue culture system.

Technique of the Test.—Suspensions of lymph node cells were prepared in such a way that the desired number of cells was obtained in 1 ml of nutrient medium. The nutrient media were removed from the Petri dish containing the 6-day-old brain cultures, 1 ml of the lymph node cell suspension was added to each Petri dish, and the cultures were returned to incubation at 37°C. One hour later, 4 ml of nutrient media were added to each Petri dish.

Microscope observations and microphotographs of the experiments were made with phase contrast optics of cells in culture in the Petri dish, on mounted coverslips without staining, and on coverslips after fixation and staining with Jacobson's stain according to methods previously described (7). First observations were made 1 hour after the lymph node cells were added to the brain culture. Eight days later observations were again made.

RESULTS

Cellular Elements of Brain Tissue Culture.—On the 6th day following the preparation of the brain cultures, the cells formed a monolayer in which a population mixture of the following elements could be observed:

1. Fibroblastic type cells were frequent, as shown in Fig. 8. They showed

well defined nucleoli, nuclear membranes, and mitochondria. Some components of the mesenchyme persisted as undifferentiated elements, showing a large cytoplasm and active undulating membranes.

2. Phagocytic cells, probably arising from perivascular histiocytes or monocytes, showing the characteristic peripheral membranes.

3. Oligodendroglia elements were easy to distinguish from the other components of the culture owing to their globose form and rich granular content which produce a halo around the perinuclear cytoplasm when observed under phase contrast microscopy (8). The processes of these cells are narrow, arising from the perinuclear cytoplasm as shown in Figs. 10, 12, and 8.

4. Astrocytes were numerous, with various and dense processes forming characteristic feltworks in some fields (Figs. 10 and 8).

5. Pial elements showing a rich internal fibrillar structure could be occasionally observed.

Behavior of Lymph Node Cells in Brain Cultures.—Within 1 to 3 hours of contact with the brain tissue cultures, the sensitized lymph node cells started to adhere to certain cell elements of the brain culture and to aggregate, forming clumps consisting not infrequently of 10 to 15 cells (Fig. 5). The reaction was most intense on the 3rd hour of contact, as shown in Figs. 1, 3, 5, 13 to 15. Cell aggregates were still present 24 hours later, as seen in Fig. 7, although fewer clumps were observed in the field than after 3 hours of contact. The agglutinated and non-agglutinated lymph node cell population gradually disappeared from the culture, so that by the 3rd to 4th day of incubation only occasional cells were encountered on the background of proliferating fibroblastic elements of the brain culture (see below).

In order to estimate the optimal concentration of lymph node cells which would agglutinate in contact with the brain culture cells, serial twofold dilutions of sensitized cells were made in nutrient medium and added to the cultures. The agglutination reaction was best observed when the concentration of cells was 15 to 30 million for each Petri dish. Cell aggregates were still formed when 2 to 10 million lymph node cells were added to each culture, but it was difficult to record the results since only a few cell clumps were found in each field of observation.

No agglutination was observed with either aggressive or normal lymph node cells placed in contact with brain cultures (Figs. 2, 4, and 16). The occasional cell clump observed did not adhere to brain cells in culture, the lymph nodes remaining essentially in a monodispersed state (Figs. 2 and 6) throughout the observation period. The aggressive and normal cells disappeared from tissue cultures much earlier than the sensitized cells.

Since the density of the lymph node cell population made it impossible to define accurately the type of tissue culture cells involved in the reaction, more detailed observations (Figs. 14 and 15) and the resulting outgrowth of cultures

consisting of fibroblasts only (see below) indicated that the sensitized cells aggregated on and adhered to the glial elements such as oligodendroglia and astrocytes and not to the fibroblasts (Fig. 15).

The agglutination of sensitized cells was not observed in the absence of brain culture cells; however, they thrived well under normal tissue culture conditions. Examination of the cultures on the 5th or 6th day of incubation revealed presence of large multinucleated cells, with round nuclei, and one prominent nucleolus. The nuclei were often surrounded by a zone of vacuoles and pinocytosis was frequently observed. It was very difficult, if not impossible, to maintain either normal or aggressive cells in tissue culture and none of the few cells which persisted in the culture showed the characteristics of the cells originally obtained from sensitized lymph nodes.

Appearance of Brain Cultures Exposed to Lymph Node Cells.—Continuing observation of cultures after the disappearance of lymph node cells showed a marked difference in composition of cellular elements of the culture exposed to sensitized cells on one hand and aggressive and normal cells on the other.

On the 6th day of incubation, the brain cultures originally exposed to sensitized cells consisted entirely of fibroblastic type cells (Fig. 11). The glial elements were gone, apparently destroyed by contact with the sensitized cells. In contrast, brain cultures exposed either to aggressive or normal cells contained the original mixed cell population consisting of glial and fibroblastic elements. The glial elements such as oligodendroglia (Fig. 12) were normal in appearance, apparently undamaged by contact with aggressive and normal lymph node cells. No difference was observed between the morphological appearance of these cultures and that of cultures which were not exposed to contact with lymph node cells (Figs. 8 and 10).

Absence of Reaction to Serum.—On occasions, rat serum admixed to the nutrient medium at 30 per cent concentration was cytotoxic for the brain cultures, destroying fibrocytic and glial elements indiscriminately. This reaction occurred regardless of the origin of the serum and was related to the individual animal donor. No differences were noted in the cytotoxicity of sera obtained from normal rats and from those exposed to guinea pig cord tissue antigen (either before paralysis or after recovery). At concentration below 20 per cent, the rat serum was rarely cytotoxic.

Organotypic Specificity of the Reaction.—In order to obtain more direct evidence that the fibroblastic elements of brain culture did not participate in the reaction, kidney tissue was removed from puppies at the same time as brain tissue and grown out in monolayers. The cell population of the kidney tissue culture was primarily of the fibroblastic type. When these and brain tissue cultures were exposed simultaneously to sensitized lymph node cells, no agglutination reaction was observed in the kidney tissue cultures whereas the sensitized cells agglutinated as usual in the brain cultures. No destruction of the kidney tissue fibroblasts was observed.

Reactivity of the Lymph Node Cells in Relation to the Time of Injection of Antigen.—Groups of rats were injected with guinea pig cord tissue and their lymph nodes harvested at different time intervals, as shown in Table I. Thirty million cells were added to each Petri dish of brain cultures and the agglutination reaction observed after 3 to 4 and 18 to 20 hours of contact. It will be noted, as shown in Table I, that cells obtained from rats 1 day after inoculation of antigen or from those recovering from paralysis 20 days after injection of antigen did not form aggregates. Cells obtained from rats 4 and 14 days after exposure to antigen (the latter group of animals being paralyzed) showed a

TABLE I

Agglutination in Tissue Culture of Lymph Node Cells Obtained from Rats at Different Time Intervals after Inoculation of Antigen

Days after antigen	Condition of the animal	Agglutination	
		Hrs. of observation	
		3 to 4	18 to 20
1	Healthy	−	−
4	"	±	+
7	"	+	++
11	Suspect	++	+++
14	Paralyzed	±	±
20	" (recovering)	−	−
Control	Healthy	−	−

−, no agglutination.
±, weakly positive reaction.
+, cell aggregates observed after scanning several fields.
++, majority of the cells in aggregate form.
+++, all cells in aggregate form.

weak agglutination reaction. Lymph nodes harvested from rats sacrificed 7 and 11 days after inoculation of antigen yielded cells which agglutinated strongly. Animals of the last group (11 days) became paralyzed a day later.

Reactivity of Aggressive Cells Exposed to Sensitized Serum.—Undiluted sera obtained from groups of animals mentioned in Table II were absorbed with packed lymph node cells obtained from rats injected with adjuvants 6 to 8 days previously. Following incubation and removal of serum, cells were resuspended in culture medium and counted, and 30 million cells were added to each Petri culture of brain tissue. The cultures were incubated at 37°C and observed for agglutination reaction. The results shown in Table II indicate that aggressive cells used in the absorption test with serum obtained from rats injected with guinea pig cord antigen 7 to 9 days before became "endowed" with the

capacity to form aggregates in contact with brain tissue culture. The reaction (Fig. 9) was first noted after a longer contact time than in the case of sensitized cells; otherwise, it was similar to the one described above even though the number of cell aggregates recorded at the peak of the reaction was lower in comparison with the sensitized cells.

Exposure of aggressive cells to normal rat serum and serum obtained from rats recovered from paralysis did not change them into reactive cells. Also, in one experiment, absorption of sensitized serum obtained 7 days after incubation of antigen failed to change the normal behavior of aggressive cells *vis à vis* the brain tissue culture.

TABLE II

Absorption of Rat Sera with "Aggressive" Cells and Reactivity of the Cells in Brain Tissue Culture

Material used for absorption	Agglutination of cells in brain tissue culture	
	Experiment 1	Experiment 2
Rat serum, normal		−
" " , recovered from paralysis		−
" " , injected with adjuvants		−
" " , " " guinea pig cord*	++	−
" " , " " guinea pig cord‡		++
Intracellular contents of sensitized cells		−

* 7 days before.

‡ 9 days before.

++ and −: see legend, Table I.

Intracellular contents of sensitized cells obtained by disruption of the cells through ultrasonic vibration were not cytotoxic for brain tissue cultures and failed to make the aggressive cells reactive following absorption.

The agglutinating properties of the sensitized cells were not influenced by contact in an absorption test with serum obtained either from rats recovered from paralysis or from animals sensitized through exposure to antigen 7 to 9 days previously.

DISCUSSION

Allergic encephalitis and other experimentally induced autoimmune diseases are caused by the emergence of an antibody directed against a target organ which is antigenically identical with the tissue used for immunological stimulation of the animal. The only technique available so far for demonstration of this antibody is the passive transfer of lymph node cells from a sensitized animal to a

normal susceptible recipient (1, 2). This is a cumbersome technique and its relative insensitivity may account for our inability to demonstrate the presence of the same antibody in serum from sensitized rats.

The present paper describes a method for detection of antibody in an autoimmune disease, based on a reaction which occurs between lymph node cells obtained from a rat sensitized against a brain antigen and brain cells maintained in tissue culture. In the reaction, the sensitized lymph node cells agglutinate on or around the glial cells of the culture which are destroyed in the process leaving a culture consisting essentially of fibroblastic elements.

Although the agglutination of cells may have a superficial resemblance to the so-called mixed agglutination reaction, which has been used successfully for demonstration of blood group antigens on a variety of cells (11, 12), or to the hemadsorption test, used for detection of virus-infected tissue culture cells (13), the mechanism of the reaction described herein is quite different, since neither serum nor virus participate in the reaction. Similarly, there seems to be little resemblance to the phenomenon of "peripolesis" described as a wandering movement of sensitized lymphocytes around macrophages (14).

Since the underlying mechanism of the agglutination of lymph node cells in brain cultures seemed to be unrelated to any known immunological reaction, it is proposed that a new name, that of "contactual agglutination" be applied to the description of this test. The contactual agglutination reaction is organotypic but not species specific, since guinea pig spinal cord sensitized the lymph node cells to react with glial cells of dog brain but not with fibroblasts.

The effect of the sensitized cells upon the brain tissue cultures does not stop at the agglutination reaction since glial elements disappear from the affected cultures. The method described above did not permit preparation of cultures consisting of neurons, myelin sheaths of which would be a logical target for the sensitized cells (15). Destruction of non-myelinated cells such as glia, however, may add another indirect support to the observation (16) that myelin sheaths may be formed by oligodendroglia which were apparently targets for the sensitized cells in the present system.

Neither the glial elements nor the fibroblasts were damaged specifically by exposure to serum obtained from either sensitized rats or those recovered from paralysis. In this serum the results differ somewhat from those obtained by Bornstein and Appel (17) who described damage of neuroglia by a sensitized serum. It was impossible, however, to check the myelin-destructive activity of the serum (16) since, as mentioned above, no myelinated cells were present in the culture.

A certain parallel may be drawn between the destruction of the glial elements of the culture as a result of an apparent contact with sensitized cells and a homograft reaction *in vitro*. References to the latter subject are limited to observations related to the effect of sensitized cells on tumor cells (18) and on

kidney cells obtained from a dog whose other kidney was used previously as a sensitizing antigen (19).

The contactual agglutination reaction and its apparent cytocidal sequelae may open new approaches to the homograft reaction *in vitro*, in which emphasis could be placed on the quantitative aspects of the reaction.

The degree of sensitivity of the contactual agglutination reaction seems to be high as indicated by the detection of sensitized lymph node cells in rats exposed 4 days previously to brain antigen. The fact that almost no cell-bound antibody was found in paralyzed rats or in those recovered from paralysis 14 to 20 days after injection of antigen confirms the hypothesis that at that time all reactive cells are probably bound to the target tissue and cannot be found in the depleted lymph node.

Perhaps the most interesting aspect of this study is the observation of contactual agglutination of aggressive cells after absorption of some factor(s) from the sensitized serum. This "circulating" factor, which remains apparently undetected in serum placed in direct contact with brain cells in culture, becomes manifest when it is adsorbed onto lymph node cells. The question which remains to be answered is whether the sensitized cells are reactive because of presence of an antibody inside the cells or because of an antibody adsorbed on their surface from the serum of the sensitized animal. The inability of the sonicated contents of disintegrated sensitized cells to react against brain cells in culture neither proves nor disproves either hypothesis since ultrasonic vibration may have damaged or destroyed the antibody present in the cells. There is a possibility, however, that the "protective" antibody which can be detected in the serum of the recovered rats (9, 10) and the disease-transmitting cell-bound antibody may indeed represent a different expression, because of sites involved, of activity of the same antibody.

Although the contactual agglutination reaction followed by the destruction of glial cells has been developed in conjunction with an experimentally induced condition, there is reason to believe that the test may find an application in other experimentally autoimmune diseases such as thyroiditis, uveitis, orchitis, adrenalitis, and nephritis (15), provided that cells grown out in tissue culture from target organs other than brain will attract the sensitized lymph node cells. If this should be the case, the application of the contactual agglutination reaction may be extended to diseases of man of suspected autoimmune etiology. Lymph node cells from patients suffering from multiple sclerosis, varied forms of idiopathic encephalitides, thyroiditis, rheumatic fever, nephritis, and other diseases should be tested for reactivity against cells obtained from target organs and grown in tissue culture. If the presence of circulating antibody is confirmed in more extensive study, a test for such an antibody in blood in the above conditions may perhaps ultimately be of prognostic value.

SUMMARY

Lymph node cells were obtained from an inbred strain of Lewis rats injected with guinea pig cord tissue in Freund's adjuvant. These cells, when added to tissue culture monolayers of puppy brain, aggregated on or around the glial elements. This reaction, called contactual agglutination, was followed by the specific destruction of glial cells, leaving cultures consisting only of fibroblasts. No such reaction was noted when lymph node cells obtained either from normal rats or those injected with adjuvant alone were used. Absorption of serum obtained from rats injected with guinea pig cord tissue by non-sensitized lymph node cells made them reactive in brain tissue culture. The contactual agglutination test seems to provide an opportunity for investigation of sensitization reaction in tissue culture systems.

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BIBLIOGRAPHY

1. Paterson, P. Y., Transfer of allergic encephalomyelitis in rats by means of lymph node cells, *J. Exp. Med.*, 1960, **111,** 119.
2. Koprowski, H., Paraf, A., Billingham, R. E., and Jervis, G. A., Etudes sur le mecanisme de l'encephalite allergique chez le rat, *Compt. rend. Acad.*, 1960, **250,** 2950.
3. Koprowski, H., Jervis, G. A., and Schoeniger, D., Influence of splenectomy upon passive transfer of allergic encephalitis in a rat, *Fed. Proc.*, 1961, **20,** 38.
4. Fernandes, M. V., and Pomerat, C. M., Cytopathogenic effects of rabies virus on nervous tissue *in vitro*, *Z. Zellforsch.*, 1961, **53,** 431.
5. Eagle, H., Propagation in a fluid medium of a human epidermoid carcinoma strain KB, *Proc. Soc. Exp. Biol. and Med.*, 1955, **89,** 362.
6. Billingham, R. E., and Silvers, W. K., Transplantation of Tissues and Cells, Philadelphia, Wistar Institute Press, 1961.
7. Fernandes, M. V., The development of a human amnion strain of cells, *Texas Rep. Biol. and Med.*, 1958, **16,** 48.
8. Pomerat, C. M., and Costero, I., Tissue culture of cat cerebellum, *Am. J. Anat.*, 1956, **99,** 211.
9. Koprowski, H., The role of hyperergy in measles encephalitis, *Am. J. Dis. Child.*, 1962, **103,** 273.
10. Paterson, P. Y., and Marvin, S. M., Symposium on Mechanism of Immunological Tolerance, 1961, Prague, to be published.
11. Coombs, R. R. A., and Bedford, D., The A and B antigens on human platelets demonstrated by means of mixed erythrocytes—platelet agglutination, *Vox Sanguinis*, 1955, **5,** 111.
12. Kelus, A., Gurner, B. W., and Coombs, R. R. A., Blood group antigens on HeLa cells shown by mixed agglutination, *Immunology*, 1959, **2,** 262.
13. Shelokov, A., Vogel, J. E., and Chi, L., Hemadsorption (adsorption-hemagglutina-

tion) test for viral agents in tissue culture with special reference to influenza, *Proc. Soc. Exp. Biol. and Med.*, 1958, **97**, 802.

14. Sharp, J. A., and Burwell, R. G., Interaction ('peripolesis') of macrophages and lymphocytes after skin homografting or challenge with soluble antigens, *Nature*, 1960, **188**, 474.
15. Waksman, E. H., Experimental allergic encephalomyelitis and the "auto-allergic" diseases, *Internat. Arch. Allergy and Appl. Immunol.*, 1959, **14**, suppl. 1, 1.
16. Hild, W., Myelogenesis in cultures of mammalian central nervous tissue, *Z. Zellforsch.*, 1957, **46**, 71.
17. Bornstein, M. B., and Appel, S. H., The Application of tissue culture to the study of experimental "allergic" encephalomyelitis, *J. Neuropath. and Exp. Neurol.*, 1961, **20**, 141.
18. Rosenau, W., and Moon, H. D., Lysis of homologous cells by sensitized lymphocytes in tissue culture, *J. Nat. Cancer Inst.*, 1961, **27**, 471.
19. Govaerts, A., Cellular antibodies in kidney transplantation, *J. Immunol.*, 1960, **85**, 516.

EXPLANATION OF PLATES

PLATE 67

FIG. 1. Aggregation of "sensitized" lymph node cells (30×10^6) adhering to certain elements of a puppy brain culture after 3 hours of contact. Photographed through a Petri dish, using a unitron microscope. × 730.

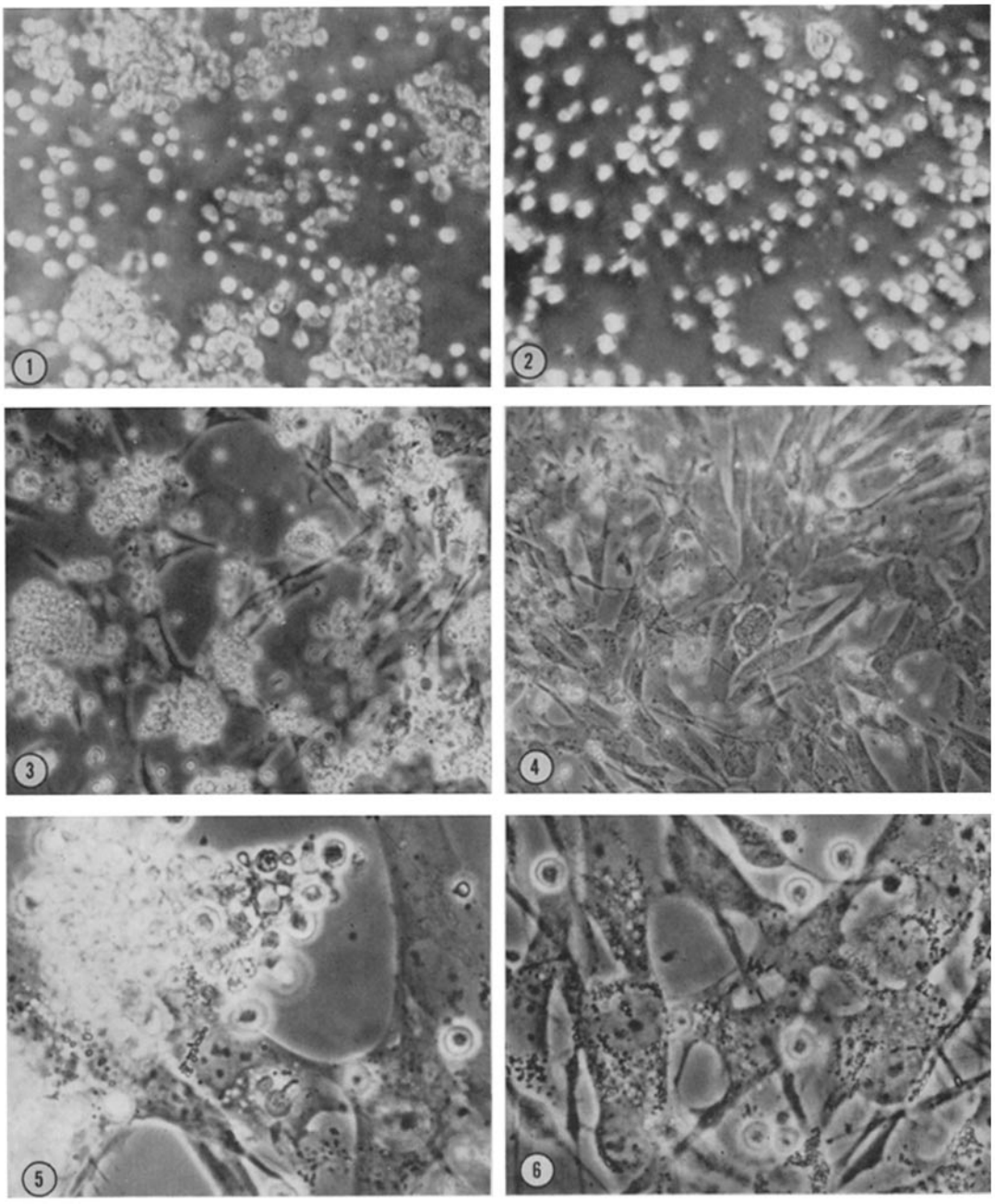
FIG. 2. "Aggressive" lymph node cells in puppy brain cultures after 3 hours of contact. Photographed through a Petri dish, using a unitron microscope. × 730.

FIG. 3. Aggregation of "sensitized" lymph node cells (30×10^6) adhering to certain elements of a puppy brain culture after 3 hours of contact. Phase contrast microscopy. × 450.

FIG. 4. "Aggressive" lymph node cells in puppy brain culture after 3 hours of contact. Phase contrast microscopy. × 450.

FIG. 5. "Sensitized" lymph node cells in contact with puppy brain cells. Phase contrast microscopy. × 1200.

FIG. 6. "Aggressive" lymph node cells in contact with puppy brain cells. Phase contrast microscopy. × 1200.



(Koprowski and Fernandes: Autosensitization reaction *in vitro*)

PLATE 68

FIG. 7. Aggregates of "sensitized" lymph node cells after 24 hours of contact with puppy brain cells. Phase contrast microscopy. × 450.

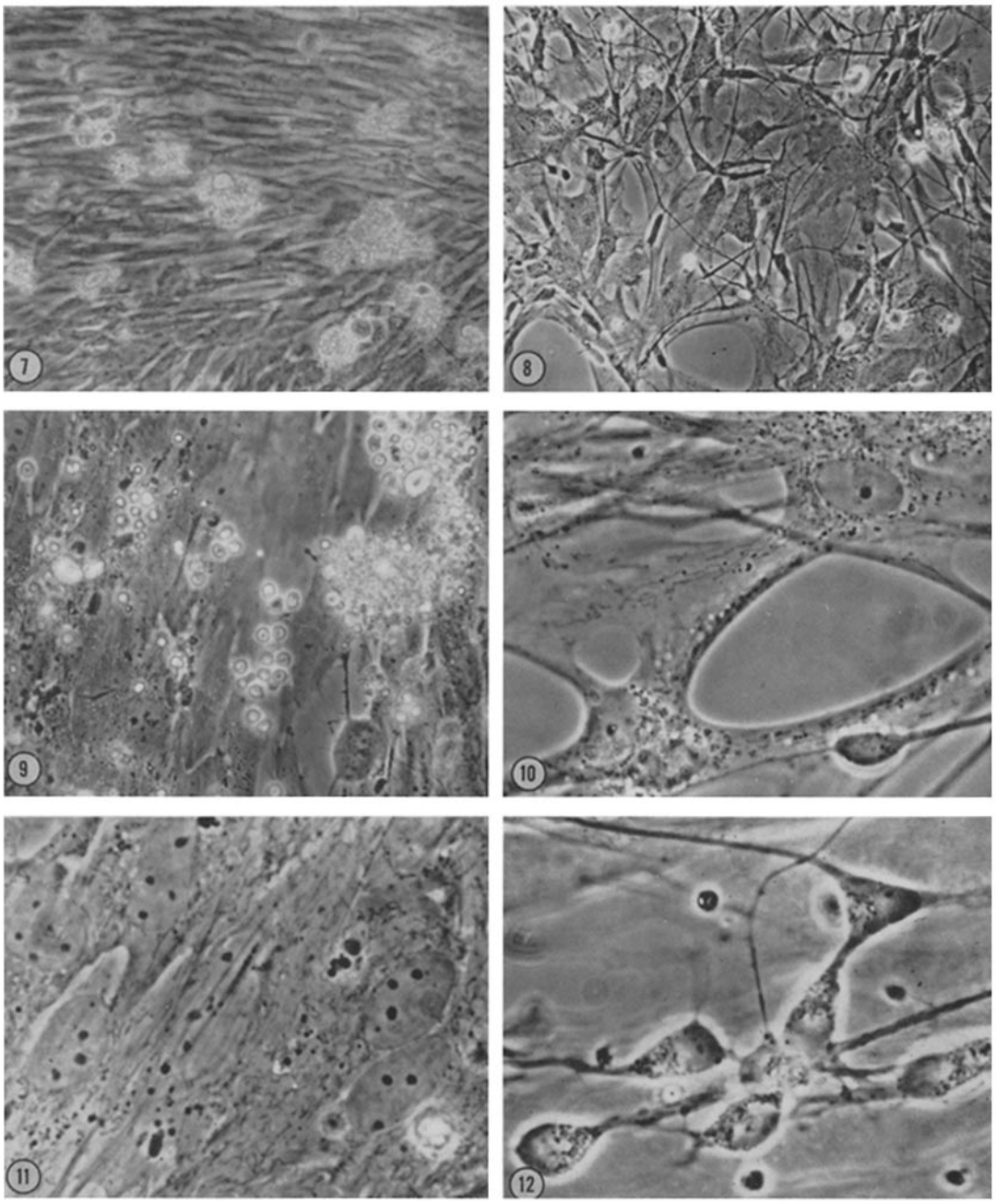
FIG. 8. Culture of mixed population of puppy brain cells. Phase contrast microscopy. × 450.

FIG. 9. Aggregates of "aggressive" lymph node cells in puppy brain culture after incubation with "sensitized" serum. See text. Phase contrast microscopy. × 450.

FIG. 10. Cultures of mixed population of puppy brain cells with typical astrocytes and an oligodendroglia element. Phase contrast microscopy. × 1200.

FIG. 11. Brain culture (all fibroblast cells) 6 days after exposure to "sensitized" lymph node cells. Phase contrast microscopy. × 1200.

FIG. 12. Brain culture with glial elements still present 6 days after exposure to "aggressive" lymph node cells. Phase contrast microscopy. × 1200.



(Koprowski and Fernandes: Autosensitization reaction *in vitro*)

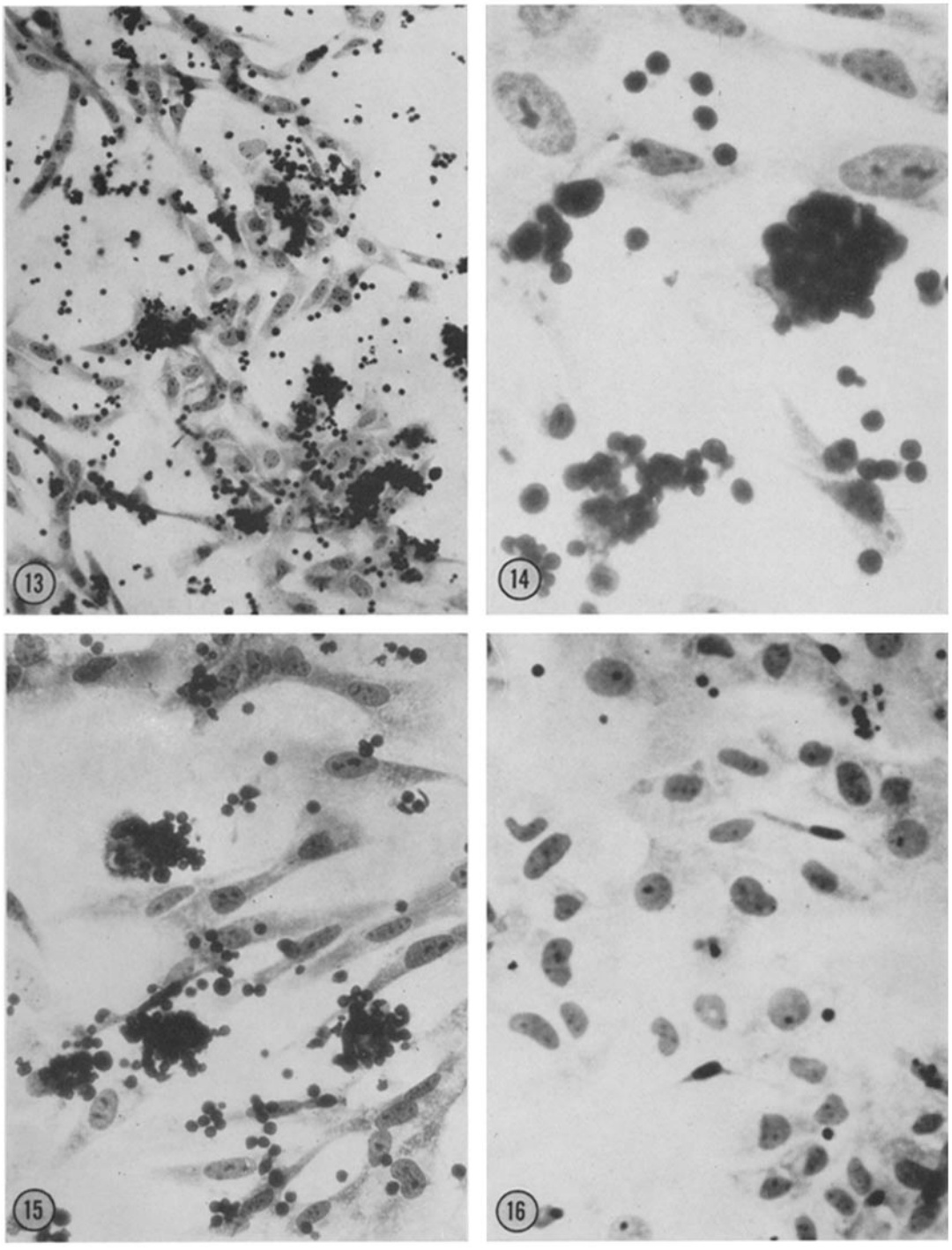
PLATE 69

FIG. 13. Puppy brain culture after 3 hours of contact with "sensitized" lymph node cells. Stained preparation. × 470.

FIG. 14. Puppy brain culture after 3 hours of contact with "sensitized" lymph node cells. Stained preparation. × 1480.

FIG. 15. Puppy brain culture after 3 hours of contact with "sensitized" lymph node cells. Stained preparation. × 740.

FIG. 16. Puppy brain culture showing glial elements after 3 hours of exposure to "aggressive" lymph node cells. Stained preparation. × 740.



(Koprowski and Fernandes: Autosensitization reaction *in vitro*)