ANTIBODY PRODUCTION BY CELLS IN TISSUE CULTURE

II. Qualitative and Quantitative Aspects of Antibody Production (Local Hemolysis in Gum) by Cells Obtained from Long Term Culture*

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(Received for publication 18 February 1966)

Most of the early studies on the synthesis of antibodies by cells in culture have been carried out with fragments of lymphopoietic organs and not with isolated cells (1, 2). More recent experiments with isolated cells have permitted the synthesis of antibodies to be studied for a short time during cell survival. However, strictly speaking, no true culture of cells was involved since the cells were not dividing in the course of the experiment (3, 4).

The formation of antibody during a true tissue culture requires two discrete events: the cellular differentiation into competent cell lines and synthesis by specialized cells. These two events do occur simultaneously in the tissue fragments and probably necessitate a minimum of tissue organization to allow expression of the cellular and biochemical interactions which appear implicated in the physiological process of antibody synthesis.

Events from this sequence in a tissue in culture have been the object of fragmentary studies related to the entry of the antigen into the competent cells, the persistence of the antigen in certain cells including the immunocytes, (5) and cellular division (6, 7). Identification of the cells involved in the production of antibodies has been performed by the use of histological techniques of detection by immunofluorescence (8, 9).

These experiments have permitted the study of the over-all production of antibodies by tissue fragments (measure of antibody titer in the culture medium) (8) and the histologic expression of this activity (number and type of cells producing antibodies at a given moment in the fragments) (9). It is a manifestation in situ of the immunological activity of tissues in culture which has thus been studied.

A recent method of tissue culture, that of Trowell (10) and Jensen et al. (11), allows the collection and study of the behavior of cells removed from a tissue containing multiplying cells. These cells undergo, under these conditions, a specific morphological evolution which has been described in the preceding article (12). We consider here the antibody-producing activity of such cells, using the method of local hemolysis in gum (13, 14).

* This work has been supported by a grant No. 62 FR 085 A2 from the Délégation Générale à la Recherche Scientifique.

Two main cellular populations can be obtained from fragments of rabbit spleen or popliteal lymph node cultivated according to the method of Jensen: (*a*) free cells staying in suspension in the culture medium and (*b*) cells which, having been liberated from the fragments, are fixed on the cover slip placed under the grid platform, and are allowed to proliferate.

It is the immunological activity of these two population types that we have been studying at the same time that we have examined the morphological aspects of these immunocytes. The active cell was detected by the method of local hemolysis.

Materials and Methods

The methods of culture are described in the preceding article (12).

Two types of cells isolated from the fragments in culture are harvested: (*a*) the free cells which have dropped to the bottom of the Petri dish, including some on the cover slip, but which have not been attached to the glass and (*b*) those which have actually become attached to the cover slip. Concerning the first type, the technique of harvest and incorporation into the carboxy-methyl-cellulose (CMC) gum is exactly the same as that which is applied to suspensions of cells from fresh organs (lymph nodes or spleen) and which has already been described (13, 14).

The cells which have become attached to the cover slip placed under the stainless grid platform are treated as follows: the cover slip is removed from the Petri dish, under sterile conditions, rinsed several times in the culture medium (ordinarily Eagle-Tris buffer, with 10% calf serum added, see reference 12) in such a way as to eliminate the cells not strongly attached to the glass, and then drained. The drained cover slip is placed on a drop of CMC (0.03 ml) containing sheep red blood cells (SRBC) and complement (see reference 13) the side of the cover slip having the cells being towards the CMC (Fig. 1). The cover slip is delicately pressed so that the gum preparation spreads out until it forms a circle inscribed in the square which is the shape of the cover slip (22 x 22 mm). The edges of the cover slip are sealed with vaseline (not with paraffin, usually hemolytic) and the slides placed in an incubator at 37°C.

Detection of Immunocytes.—After being incubated overnight, which has been shown to be sufficient time for all cells capable of producing antibody to express this capacity, the cells are examined through an inverted microscope at low power (objective × 2.5) and by dark field to count the number of plaques of hemolysis and to locate their positions. Each plaque (or group of plaques when they are confluent) is marked on the external surface of the cover slip by a circle traced with indelible ink.

The cover slips are next carefully loosened from the slide and immersed in the culture medium for about a half hour so as to dissolve the CMC adhering to the cover slip. The cover slips are next rinsed with medium without serum and then fixed and stained for histological examination. In the work reported here, we have used May-Grünwald-Giemsa stain. Then one can examine the cells which were found in the center of the plaques of hemolysis (plaque-forming cells or PFC) and which are now in the center of the circles, although often one notes the presence of several cells in the circle delineating the zone of hemolysis.

We have considered in this paper only cells isolated in the zone of hemolysis, because there could be no doubt that they were producers of antibody.

Collection of Cells.—The collection of the cover slip situated under the culture platforms was carried out according to one of two schemes. Either the cover slips are collected after a constant and fairly long time period, for example every third day from the beginning of the

culture: the grid is transferred to a new Petri dish containing a clean cover slip. Or else the grid can, after a certain time in culture (e.g. 8 days), be transferred to a clean cover slip for a short period of some hours. In this last case, it is necessary, after having removed the culrure platform from the Petri dish containing the now briefly incubated cover slip, to leave this Petri dish for a certain supplementary time under culture conditions to allow the cells, which fell during the short time the fragments were present, to attach to the glass. This avoids subsequent loss of these cells on rinsing the cover slips. The two types of collection do not give results (in number of PFC) as different as those which could be expected in the first analysis, when the total quantity of harvested cells differs greatly from one case to the other. This probably means that the production of antibody by one specialized cell is only an act of short duration (of the order of 12 to 24 hr) effected by only a fraction of cells in a population. In these methods it is useless to have on a cover slip a large number of cells collected over a fairly long time, because all the cells attached in the beginning of the culture will have already expressed their capacity for synthesis before the collection and will be inactive by the time of mixture with the indicator system for PFC.

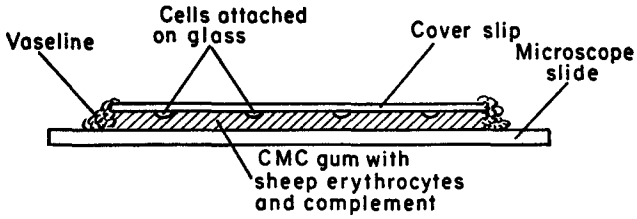

FIG. 1. Diagrammatic representation of a slide with CMC gum ready for the detection of plaque-forming cells (PFC) attached on glass after culture.

Measure of the Activity of a Population.—The number of active cells (immunocytes) is at least equal to the number of hemolytic plaques and it would be greater in the case where more than one cell participates in the formation of a plaque. This latter possibility has been eliminated by an experiment with suspensions of cells taken from organs of immunized animals and incorporated immediately into the indicator system. In such case the number of PFC is linearly related to the number of leucocytes per milliliter in a given preparation.

With tissue culture cells (attached on glass) we have no way to apply this dilution test, therefore we cannot bring a direct experimental proof that one plaque is due to one cell only, we are thus obliged to rely on inference based on the behavior of cells from suspensions, which, in our opinion, bring rather strong evidence for the "one plaque–one active cell" idea.

It is necessary, on the other hand, to count the total number of attached cells on each cover slip in order to express the activity of the population. For this we have used three procedures: (*a*) counting of nucleated cells in the hemolytic plaques (clear zones) followed by the calculation of the ratio of the surface of hemolytic zones to that of the whole preparation, (*b*) counting by "random" sampling of white cells after lysis in situ of the red cells by rapid freezing and thawing of the whole cover slip, (*c*) counting (also by sampling) of all the cells attached to the cover slip after washing out the gum, fixing, and staining. This last procedure has one drawback: in the course of eliminating the CMC gum by dissolution, some white cells can be detached from the glass.

The results of these various determinations will be discussed later, but they have given values, in general, of comparable orders of magnitude. However, the second method which ought to give the results nearest to reality, since it takes into account the real distribution

of cells on the cover slip, always gives lower values than those furnished by the first procedure. This can be explained by the fact that, with method (*a*) one postulates that the mean density of the population of cells on the complete cover slip is the same as that which is measured in the hemolytic zone formed around PFC, which is evidently incorrect and furnishes figures erroneously high and consequently low activity values.

In a recent experiment in which we have been able to compare the values obtained using methods (*a*) and (*b*) on the same preparations, we have ascertained that the second gives, on the average, values two to three times lower than the first. Consequently the results concerning the number of cells harvested (and the activity of the preparation) indicated in the tables which follow should be considered as orders of magnitude and more than likely much higher than the real values.

RESULTS

The hemolytic plaques are due to an immunological phenomenon because their production necessitates the presence of complement (in the form of fresh guinea pig serum) and the culture cells coming from tissue of normal (non-immunized) animals never give centers of hemolysis. In this sense the background "noise" (number of PFC) of culture cells attached to the cover slips appears weaker than that of normal rabbit lymph node or spleen cells suspensions, but this may be an artifact due to the small number of cells examined. Each cover slip examined contains a maximum of only 10^4 to 10^5 cells, while the activity of a suspension is calculated after an examination of at least 2×10^6 cells.

The study of the growth of hemolytic plaques formed around the culture cells provides further evidence that these cells synthesize antibody. In fact the increase of the diameter of these plaques follows Fick's law (Fig. 2); i.e., is proportional to the square root of time. This agreement is observed over fairly long time periods (at least 340 min). In contrast, if experiments are performed with paper strips on which rabbit anti-SRBC hemolysin has been absorbed it is found (Fig. 3) that the width of the zone of hemolysis increases, in the course of time, also following Fick's law. However the curve deviates from that of Fick, 100 min after the start of diffusion; at this time the volume of the zone of hemolysis corresponds to 25% of the reservoir's volume. Consequently, the growth of the lytic plaque follows Fick's law only until the concentration of antibody in the reservoir has dropped to 75% of initial value. This is very understandable, for the calculations from which Fick's law is derived postulate that the concentration of the reagent in the starting reservoir does not change significantly in the course of the diffusion, which clearly cannot be true for a long time in this type of experiment.

Now, in the lytic plaques generated by the cells in culture, the growth law follows Fick's law for relatively longer periods of time, for example 340 min (Fig. 2).

At this time the volume of the lytic zone is equal to at least *100 times that of the cell*. It is impossible to relate this phenomenon to simple diffusion of anti-

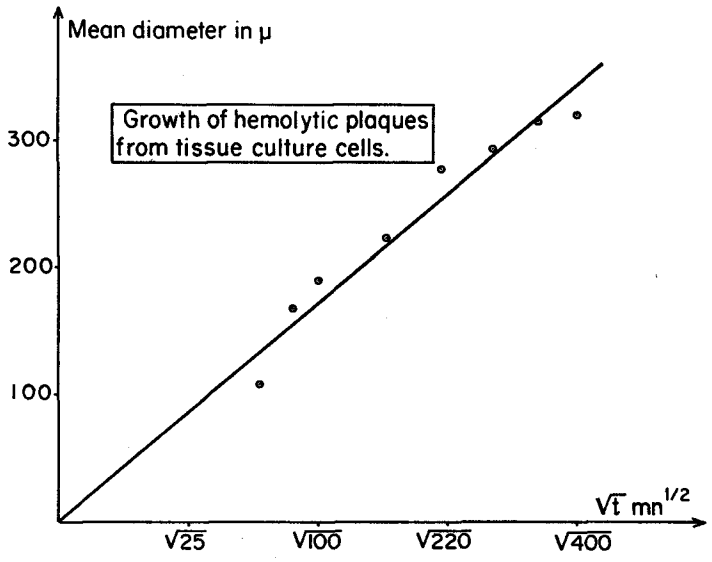

FIG. 2. Growth of plaques of local hemolysis in gum produced by tissue culture cells attached on glass.

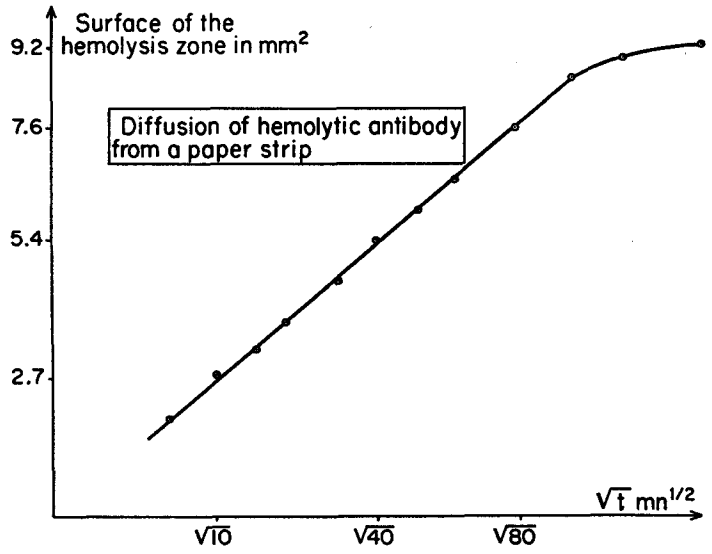

FIG. 3. Growth of a zone of hemolysis in gum produced by diffusion of antibody from a paper strip.

body from a finite reservoir, even if it were the entire volume of the cell (of the order of 10^{-9} cm^3). It is necessary, then, to conclude that the phenomenon of plaque formation is due to an active synthesis of antibody by the cells attached on the cover slip placed on the gel; this synthesis must last at least 5 hr and probably much longer. In fact the study of the growth of plaque size during 24 hr shows that many plaques increase between 7 and 18 hr after the contact of cells with the CMC indicator preparation.

A study by microcinematography of the PFC derived from cellular suspensions has shown us that the synthesis of antibody by PFC corresponding to a continuous growth of lytic plaques is always accompanied by fairly intense

TABLE I

Cell Types and Immunological Activity (PFC) of Free Cells Obtained with Different Types of Culture

	Free cells in suspension				Cells from tissue fragments			
	At beginning		After 24 hr		After 3 days		After 7 days	
	Nodes	Spleen	Nodes	Spleen	Nodes	Spleen	Nodes	Spleen
Viable cells, %	66	68	83	75	57	64	76	50
Differential count								
Lymphoblasts	4	1	4	0	0	—	0	0
Prolymphocytes	47	23	6	7	21	—	8	12
Lymphocytes	45	73	90	33	79	—	89	88
Plasma cell	4	3	0	0	0	—	3	0
No. of PFC/10^6 cells	1020	200	16	22	14	1	39	13

cellular movements on the surface and in the cytoplasm. The microcinematographic examination of culture cells shows equally well that the PFC are always in movement during the period of plaque growth. This is a proof that the PFC from our cultures are alive during all the duration of increase of the plaque size.

A first experiment using a culture of a week's duration (Table I) has permitted us to compare the behavior of population of free cells or of cells stuck to the cover slips.

Cells obtained from the popliteal lymph nodes and spleen of secondarily stimulated rabbits, which have received a booster intraplantar injection 4 days before sacrifice have also been studied. Two types of culture have been used: (*a*) a suspension of free cells is placed into a Petri dish at rest or (*b*) fragments (1 mm^3 on the average) are placed on grids or on the bottom of the Petri dish. The evolution of cell types in the culture (differential counts) has been described in the first article of this series.

The immunological activity of suspensions of free cells (number of PFC per 10^6 cells) declines more rapidly than the viability after 24 hr of culture; the former is no more than 1% of the initial activity for spleen cells suspensions while the latter remains relatively high. After 9 days, the immunological activity is very weak for lymph node cells and nil for spleen cells.

TABLE II

Immunological Activity (PFC) of Cells from Organ Culture

Cells attached on glass and free cells.

	3rd day		7th day	
	Nodes	Spleen	Nodes	Spleen
Cells attached on glass				
No. of cells	40,000	—	33,000	83,000
No. of PFC	3	0	37	3
PFC/10^6 cells	75	0	1110	36
Free cells				
No. of cells × 10^6	4.3	3.7	5.8	3.3
Viable cells, %	57	65	76	50
PFC/10^6 cells	14	1	39	13

Immunological activity of cell suspensions at the beginning of culture:
= nodes, 1030 PFC/10^6 cells
= speen 200 PFC/10^6 cells

Differential count:	Nodes, %	Spleen, %
Lymphoblasts	5	0
Prolymphocytes	35	14
Lymphocytes	26	80
Plasma cell	34	6

On the other hand, with cell cultures, though the number of active cells collected in 3 days is not considerable (Table II) then, after 7 days of culture, the number of active cells is fairly large.

The activity (per 10^6 cells) of attached cells originating in lymph node fragments is higher than that of the population of free cells. Yet the total number of attached cells after 7 days (30,000 at the most) is much smaller than that of the free cells (of the order of 6×10^6). The fixation on glass must then effect a selection of antibody-producing cells.

Another series of experiments (Table III) on a culture incubated for 5 days and having its cells harvested at very different intervals has given interesting results. It can be seen that fairly long collection intervals (48 to 72 hr), beginning on the first day of culture, do give a population of fixed cells not much more

numerous than the population fixed in 24 hr. Among the cells which are liberated in the beginning of the culture, many are damaged or come from the cut edge. The cells which really migrate do not appear until later.

The mean number of cells harvested in 24 hr does not vary, however, within large limits when approximately the same quantity of lymph node tissue is placed on the platforms (4 fragments, each of about 1 mm^3). If one compares these results with those of Table II, where the cells formed by the fragments in 7 days and kept free in the medium have been counted, one could have an idea of the yield of "cellular production" of fragments. If one assumes that a frag-

TABLE III

Number and Activity (PFC) of Cells Harvested from Organ Culture

5 days' culture.

	Collection intervals, *hr*					
	0–24	0–48	48–64	0–72	100–104*	104–120
Cell No. <	72,500	59,000	41,000	94,000	15,200	35,500
No. of PFC per cover slip	51	3.5	8	3	1.7	52.5
PFC/10^6 cells >	701	59	195	32	112	1,480
No. of cells attached per day	72,500	29,500	61,500	31,000	91,200	53,000

Cell suspension from the lymph nodes at the start of the culture.

Immunological activity: 800 PFC/10^6 cells

Differential count: %

Lymphoblasts 0

Prolymphocytes 19

Lymphocytes 69

Plasma cells 12

* A further 16 hr were allowed for the cells to attach on glass after removal of the platforms bearing the fragments.

ment of 1 mg (1 mm^3) contains about 5×10^7 cells, the results of Table II indicate that one can obtain 2% of this number of cells in the form of free cells after 3 days of culture. From these free cells, 1% fix on the glass.

We have noted that usually after the first important phase of activity, for the cells collected in the course of the first day of culture, the activity of the harvested populations falls, only to rise again in a week. This early phase of activity perhaps corresponds to the activity of harvested lymph node tissue being of the same order of magnitude as that of the cell *suspension* prepared from the same lymph node at the beginning of the culture of the tissue fragments. The PFC fixed on the cover slips after the first day of culture are perhaps cells related to the PFC of the suspension made at the start of the culture. Differentiation may perhaps take place afterwards, in the culture fragments, so that a new genera-

tion of PFC is expressed belatedly, for example 1 wk after the start of culture.

The last two colums of Table III clearly show the effect of conservation of isolated and fixed cells on the glass, on their immunological activity: in the next to the last column (100–104) the cover slips have been collected after 4 hr under the platform, cultivated for a further 16 hr in fresh medium alone and placed on the CMC to allow the PFC to be counted. In the last experiment (104–120) the cover slips were left 16 hr under the fragments and then, after washing, immediately placed on the gum. In this case, the immunological activity of the population is much greater than in the preceding case, which indicates that many cells have, in the course of 16 hr in the Petri dish, lost their synthesizing capacity (perhaps simply because they died). These findings are entirely in agreement with what we find with the cell suspensions prepared from lymph nodes or spleens of immunized animals. Consequently, one can conclude that immunocytes, by whatever procedure they are obtained, have only a limited period of activity ended by their disappearance.

In the course of the collection of cells from fragments there is, at any given time, a fraction of the population engaged in the process of antibody synthesis, a fraction which is relatively constant for several weeks. For each cell the productive period would be fairly short compared with the period of incubation of cover slips under the fragments and of such a nature that, if one removes the fragments from the Petri dish, just leaving the cover slips a certain time in the medium, it allows the greater part of cells capable of producing antibody to express this capacity before being incorporated into the gum. There exists, then, at the time of contact of the cover slip and the gum, only a population of weak activity.

Culture experiments of longer duration (2 wk) has confirmed this view (see Table IV). During these experiments the number of cells attaching on glass did not increase regularly although the relative activity of the population was increasing. This may be the result of some unknown changes in the tissue culture conditions but could also occur by renewal of the cells attached to glass.

The results of an experiment with cultures of popliteal node fragments followed for more than 3 wk are reported in Table V. It can be seen that the number of cells obtained in 3 to 4 day intervals varies markedly, with the maximum yield occurring during the second week. There is, afterwards, a decrease which may correspond to an exhaustion of the productive capacity of the fragments in free cells. With respect to the PFC, an increase in the over-all activity of the population of the fixed cells can be observed till the 5th day. This activity is, as we have seen before (Table II), generally greater than the activity of the population of free cells from the same fragment (though this population is very numerous). It is, in fact, larger than the activity of the cell suspensions prepared from lymph nodes in the beginning. From the end of the

TABLE IV

Number and Activity (PFC) of Cells Harvested from Organ Culture

2 wk of culture.

	Collection intervals, *days*											
	0–7			7–8			9–10			10–14		
	G1	G3	G5	G1	G3	G5	G1	G3	G5	G1	G3	G5
No. of cells <				62,000	7,000	10,000	11,000	15,000	—	20,000	—	—
No. of PFC per cover slip	158	116	100	23	5	4	13	2	0	3	0	0
No. of PFC/10^6 cells				370	707	388	1,200	134	0	153	0	0

Cell suspension from the lymph nodes at the start of the culture.

Immunological activity: 390 PFC/10^6 cells

Differential count: %

Lymphoblasts 0

Prolymphocytes 18

Lymphocytes 73

Plasma cell 9

TABLE V

Number and Activity (PFC) of Cells Harvested from Organ Culture

3 wk of culture.

	Collection intervals, *days*						
	0–2	2–5	5–8	8–12	12–15	15–20	20–25
Cells attached on glass							
No. of cells <	9,000	15,300	50,000	34,000	61,000	15,000	5,000
No. of cells per cover slip	14	29	21	23	10	5	2
PFC/10^6 cells >	1,540	1,900	420	673	164	325	420
Free cells							
No. of cells per Petri dish × 10^6	1.64	0.4	0	—	—	—	—
No. of PFC/10^6 cells	65	180	0	—	—	—	—

Cell suspension from the lymph nodes at the start of the culture.

Immunological activity: 555 PFC/10^6 cells

Differential count: %

Lymphoblasts 1

Prolymphocytes 21

Lymphocytes 70

Plasma cell 8

first week of culture the immunological activity of the population of attached cells levels off at a mean value of a few hundred PFC per 10^6 cells. This activity is stable up to the end of the experiment (25 days); the last collection (cells harvested between the 20th and 25th day) has an activity which is of little significance, as the number of PFC is too small. This experiment shows that it is possible to keep reticuloendothelial cells from an immunized animal in culture and that these cells express their immunological activity for a period of about one month without any further antigenic stimulation on the tissue in

TABLE VI

Number and Activity (PFC) of Cells Harvested from a Two Weeks' Organ Culture

Lymph node collected 1 yr after SRBC injection.

	Collection intervals, *days*				
	0–2	2–6	6–9	9–13	13–16
Cells attached on glass					
No. of cells	17,400	18,400	17,800	46,000	23,200
No. of PFC	0.66	4.4	10	13.2	4.2
PFC/10^6 cells	38	240	565	287	180
Free cells					
No. of cells per Petri dish	1,700,000	410,000	340,000	250,000	0
PFC/10^6 cells	1.7	43	107	9	0

Cell suspension from the lymph nodes at the start of the culture.

Immunological activity: 1.6 PFC/10^6 cells

Differential count: %

Lymphoblasts 1

Prolymphocytes 25

Lymphocytes 63

Plasma cell 11

culture being necessary; this activity is unlikely to be due to the cells which were active at the beginning of the experiment.

Another type of experiment has been performed with lymph nodes taken from a rabbit a considerable time after immunization by a local injection of SRBC but not reimmunized for the preceding year (Table VI). At the beginning of the experiment the cell suspension from the lymph node fragments does not express any immunological activity (1.7 PFC per 10^6 cells, activity close to the "background" found in most normal rabbits). After 48 hr of culture, the number of cells fixed on the cover slips is small (less than 17,400) but a modest activity can be seen. On the other hand, the free cells, which are rather numerous, have activities no different from those in the initial suspension.

During the culture, the daily output of cells attaching on the glass is constant but small (from 5 to 10,000), in fact much lower than the number which can be collected from recently stimulated nodes (Table III). On the other hand the immunological activity of the cell population increases up to 9th day of culture to reach a value of 565 active cells/10^6, of the same order as the activity found in a lymph node from a rabbit in a stage of secondary reaction.

The population of free cells also shows an increase of activity reaching 107 PFC/10^6; this activity then decreases rapidly although the activity of the fixed cells decreases only slowly; this latter being still 180 PFC/10^6 up to the 16th day of culture.

The result of this experiment started with a view of testing the possibility of inducing a secondary stimulation in vitro is interesting as it indicates that, without any new stimulation, cells of an animal in immunological rest can, by simple tissue culture, start to manufacture antibody. Different interpretations of such a phenomenon can be suggested and will be discussed later but the occurrence of such behavior is of interest.

DISCUSSION-CONCLUSIONS

The results mentioned here show that it is possible to maintain for a few weeks a culture of reticuloendothelial organs from an immunized animal and to collect from this organ free cells during the whole culture period, the majority of which stay free and some of which attach on the glass. These two populations can express immunological activity, that of the attached cells being more prolonged. In any case the two populations show the persistence of immunological activity much longer than that expressed by suspensions of free cells cultured in Petri dishes after mechanical extraction from the node or spleen.

The reason for this behavior is probably that the method described here provides a method to obtain immunologically mature cells after division of the stem cells present in the fragment. Furthermore, there is probably a selective pressure in favor of the immunocytes: it is generally admitted that an antigenic stimulus increases the division of the cell precursors of the antibody-forming cells. This dividing capacity helps the cells to escape from the fragments, to go across the paper and the grid, and finally to fall to the bottom of the Petri dish.

When the period of culture exceeds 1 wk, no free cells coming from the organs can be found in the medium, though the cells attached on glass can be harvested for more than 3 wk.

As for the immunological activity of the cells, it is known that free cell suspensions are very sensitive to culture conditions and that their activity declines within a few hours, in vitro. On the other hand the stem cells present in the fragments are in more favorable living conditions and keep their potentiality for a long time. These stem cells, instead of falling themselves, do transfer the synthesis capacity to their daughter cells attaching on the glass.

Nevertheless, when the immunocytes have attached on glass, they can syn-

thesize antibodies for only a relatively short time and apparently are not able to divide and give birth to a new population of immunocytes. So we think that it is the physiological conditions inside the fragments which can explain the favorable results we have obtained, in terms of immunological capacity.

These considerations apply to organs from animals during a secondary stage. Organs from animals in immunological rest behave differently: the free cells show an immunological activity which instead of decreasing fast, as is the case for the first type of population, increases, to reach a maximum between the 6th and the 9th day.

The physiological behavior of cells stimulated in vivo is consequently quite different from the behavior of cells which undergo spontaneous immunological reviviscence in vitro. The true explanation of such a spontaneous development of PFC by simple culture of a tissue from an animal in immunological rest is not known of us; different interpretations can be given. For instance it can be supposed that the culture medium contains a substance which has a cross-antigenicity with the SRBC; calf serum for instance. However, none of the immune sera from rabbits injected with SRBC gave any reaction by ring-test with the tissue culture medium used in our experiment. If a "cross-stimulation" is not involved one may imagine a kind of spontaneous derepression of the immunological potentiality from a tissue as soon as this tissue is under culture conditions. If such is the explanation it will be of great interest for the immunologist.

Finally a last explanation has been suggested to us by the results of recent experiments in which we have found that it was possible to stimulate in vitro uncommitted cells from no immunized animals: Could it not be that some of the cells attached on glass are boostered, when in the gum, by the sheep erythrocytes and start producing antibodies?

We believe that the method described here provides a tool for the study of the sequence of the immune response under conditions which are simpler to analyze than the in vivo systems.

As the tissue is kept in vitro it is possible to control the conditions of the medium and allow the study of the influence of the antigen and of different pharmacological substances (antimitotics, amino acids, analogues, and other specific inhibitors of metabolism). It may also allow a study of cellular division and of cell filiations by the use of radioactive nucleic acids precursors such as thymidine.

SUMMARY

By combining a tissue culture method with the detection of antibody-producing cells by local hemolysis in gum it has been possible to follow the immunological activity of cells from tissue fragments for long period of time.

These fragments were obtained from lymph nodes or spleens of rabbits immunized by sheep erythrocytes.

It was found that, while the immunological activity of the free cells in suspensions decreased fast and disappeared in a few days, the cells attaching on glass could express their activity for at least 3 wk. It is assumed that these cells are the daughters of cells from the fragments which were not active antibody producers at the beginning, but differentiated, during the culture, into cells endowed with two capacities: glass adherence and antibody synthesis.

One can further admit that the type of culture employed exerts a selective pressure favoring formation of antibody-producing cells.

We wish to thank Mr. J. C. Mazie for his extremely skillful technical assistance and Dr. J. Davies for his efficient help in the translation of this paper.

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