of arterial oxygen and carbon dioxide pressures in another cat. In the first experiment (response to the left) the animal rebreathed from a bag filled with approximately 300 ml of pure oxygen. If the changes in bag volume had been recorded during this experiment, these tracings should have given information on oxygen consumption, carbon dioxide production, lung volume, and distribution of carbon dioxide in the body. The second response in Fig 2*C* (to the right) was produced by administration of a breathing mixture containing 8.6 percent carbon dioxide in air. Alveolar carbon dioxide pressure is seen to rise to 60 mm-Hg, a figure that comes close to the carbon dioxide pressure of the inspired air saturated with water at 37°C. Due to hyperventilation caused by increased carbon dioxide pressure, arterial oxygen pressure rose from 100 mm-Hg to 130 mm-Hg which, considering the reduced oxygen pressures in another cat. In the first indicates a more than tenfold increase in alveolar ventilation.

In conclusion, it may be stated that (i) a technique of gas sampling through a membrane at the tip of an evacuated cannula enables the investigator to collect gases for analysis from locations in the living body that were inaccessible to existing equipment, (ii) a complete, instantaneous, and continuous analysis of the collected gases can be obtained by connecting the cannula to a mass spectrometer, and (iii) the quantities of gas needed by the mass spectrometer are so small that gas pressures can be measured in fluids such as circulating blood, and presumably in tissues. The recordings show excellent stability and reproducibility. The membrane itself is the most critical part of the application. Further studies are needed to clarify such problems as change in permeability upon immersion of the membrane in water, effect of temperature on permeability, and the impairment of permeability in native blood which necessitated the use of anticoagulants. Attempts to use biological membranes such as frog skin, omentum, pericardium, or dura mater, have so far failed due to high water permeability.

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7. Supported by NIH under grant GM-09034.

31 May 1966

Antibody Formation in Nonimmune Mouse Peritoneal Cells after Incubation in Gum Containing Antigen

Abstract. *Peritoneal cells from normal mice in a semisolid medium containing sheep erythrocytes were incubated at 37°C for 2 or 3 days. During this period, hemolytic antibodies developed spontaneously. Arguments are presented that true* de novo *synthesis of antibody has taken place in previously uncommitted cells.*

Stimulation in vitro of normal cells by an antigen, in order to induce antibody production in vitro by these cells is a traditional aim of immunologists. Attempts in this direction have been more or less successful (*1*) but the best positive results have yielded only a very small amount of antibody. New methods for the detection of antibody formation by single cells (local hemolysis in gum) (*2*) provide a tool well-suited to the study of the aforementioned problem.

We tried first to stimulate peritoneal cells by incubating these cells with sheep erythrocytes (SE) in liquid medium prior to their incorporation in a gum of carboxymethylcellulose (CMC) containing SE as a detecting medium for hemolysin production. It soon became apparent that prior incubation in vitro of the peritoneal cells resulted in a rapid decrease of their viability index and in a poor yield of plaque formation by the mixed population (peritoneal cells and spleen cells) in the gum. Thus we have investigated the behavior of mixed (either spleen or lymph node cells added to peritoneal cells) or pure (spleen, lymph node, or peritoneal cells alone) cells immediately incorporated, after their collection, in the test system (CMC + SE + complement); we followed the events in the system for 3 days at 37°C (*3*).

We used CBA mice, 8 weeks old. Peritoneal cells were collected either immediately or 5 minutes after the injection of 0.2 ml of Hanks medium. Peritoneal cells were collected by washing the peritoneum with 3 ml of Hanks medium; cells from five mice were pooled for each experiment. Cells were counted and differential counts were established. Macrophage-type cells (highly polymorphic) amounted to about 40 percent of the population, the 60 percent remaining being of the lymphocytic type.

These cells, after one washing in an Eagle buffer, were incorporated, usually at a concentration of 5×10^6 cells/ml, in a gum of 2.5 percent CMC in Eagle-tris buffer containing 5×10^8 sheep erythrocytes per milliliter and 10 percent fresh guinea pig serum (*3*). A known amount (usually 0.022 ml) of this mixture was placed on microscope slides under a coverslip sealed with vaseline, and the preparation was incubated at 37°C. Ten preparations were examined for each experiment. At intervals the plaque-forming cells (PFC) were counted under low-power, dark-field microscopy, the results being ex-

Table 1. Spontaneous formation of antibody (expressed as PFC) by normal peritoneal cells in carboxymethylcellulose (CMC) containing sheep erythrocytes (SE).

Mode of cell collection		No. PFC/10^6 cells after		
Time after injection of Hanks solution (min)	Treatment of cells	3 hr	18 hr	45 hr
CMC with SE and complement				
0	None	0.9	23	205
5	None	0.9	32	133
5	None	0.9	3.6	6.4
5	None	0.9	94	566
5	None	0	71	328
5	Heat	0	0	0
CMC containing 10^{-3}M DNP				
5	None	0	0	0
Incubation in CMC at 47°C				
5	None	0	0	0
CMC containing actinomycin (2μg/ml)				
5	None	0	0	0
CMC with SE without complement				
5	None	0	0	0

pressed as the number of PFC per 10^6 cells screened.

Results of such experiments are reported in Table 1. In all cases the population of peritoneal cells alone shows antibody formation (though there is a great individual variability) but only after a certain lag period, namely about 15 hours as judged from other experiments. The activity is greater than the "background" activity of spleen cells of nonimmunized rabbits (1 per 10^6 cells; see *3*).

Peritoneal cells heated for 30 minutes at 60°C are inactive. Incorporation of heat-inactivated complement in the gum instead of active complement brought complete suppression of plaque formation. When inhibitors, such as dinitrophenol or actinomycin, were incorporated in the gum, plaque formation was also suppressed, although it did not interfere with the immune hemolysis itself, as controlled by separate model experiments (diffusion of hemolytic serum from a paper reservoir).

A comparison of the behavior of spleen cells, lymph-node cells, and peritoneal cells alone or mixed is reported in Table 2. While lymph-node cells yielded neither immediate response nor delayed response, the spleen-cell population did show in certain cases, and only after a certain lag period, a modest amount of plaque formation.

In mixed populations, peritoneal cells apparently play the essential part, and the presence of other types of cells does not increase the activity of the whole population.

The main problem in interpreting such data is to know whether one is observing a true *de novo* sensitization of uncommitted cells or dealing with a kind of booster stimulation. The antigen used here being a very common one, a previous encounter of the cell donors with such an antigen (or a cross reacting one) cannot be excluded. In favor of the first alternative are the following considerations.

Table 2. Antibody formation (PFC) by nonstimulated lymph node (LN), spleen, peritoneal (P) cells, or a combination, incubated in carboxymethylcellulose (CMC).

Type of cell	Number of PFC/10^6 cells after 3 hr	18 hr	45 hr
GMC with SE and complement			
Spleen	0	1	1
Spleen	1	8	13
Spleen + heated P	1	2	20
LN	0	0	0
LN + heated P	0	0	0
Spleen + P	3	26	224
LN + P	2	18	167
CMC with SE without complement			
LN + P	0	0	0

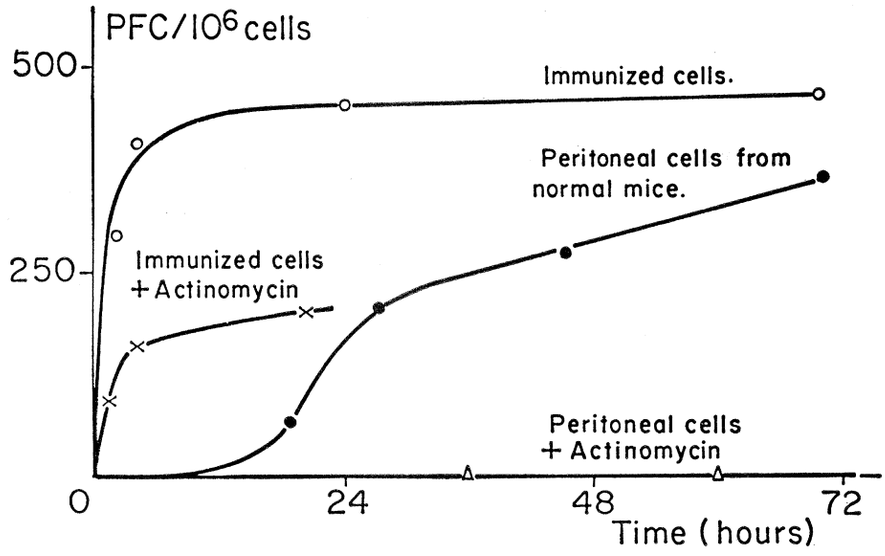

Fig. 1. Kinetics of plaque formation by lymph node cells from immunized rabbits or by peritoneal cells from normal mice.

1) If we compare the kinetics of plaque formation by lymph node cells from immunized rabbits or by the peritoneal cells of our nonimmunized mice we see (Fig. 1) that the population of immunized cells reacted rapidly and reached a maximum in 5 hours, at which time the unstimulated cells have not, as yet, reacted at all.

2) A specific inhibitor of information transcription, such as actinomycin, not only decreases the expression of plaque formation by the immunized cells but also completely suppresses plaque formation in the population of peritoneal cells from normal mice. This can be explained by the presence of a pool of specific RNA messenger for antibody to SE in the immunized cells, these cells thus being able to express antibody formation for a certain period. For the unstimulated cells, antibody formation would require synthesis of a newly formed messenger, a synthesis which is blocked by actinomycin.

3) Experiments on cell dilution with the two types of populations (immunized and normal cells) showed that, while the expression of plaque formation by stimulated cells followed a first-order curve (meaning that there is no interaction between cells required for plaque formation), unstimulated cells behave differently. In the case of unstimulated cells the number of PFC is not proportional to the concentration, but is closer to a second-order curve. This behavior could indicate that more than one cell is involved in the sequence of events leading to a true *de novo* immunization of normal cells.

We believe that our success is related to the type of medium used for the incorporation of the cells, the CMC being apparently an excellent support for keeping cells in good physiological condition for relatively long periods of time. We assume also that free, mobile cells, such as cells from peritoneal exudates, are better adapted to withstand unusual conditions of life, such as those in vitro, than cells like spleen or lymph node cells from partially fixed populations. Finally the positive results reported here may be related to the use of a heterogeneous population containing, for instance, cells from the lymphocytic series. This would be in line with the hypothesis that antibody formation requires the sequential participation of two types of cells: a phagocytic cell and an antibody-producing cell.

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4. I thank Miss E. Lachoux and Mr. J. C. Mazie for technical assistance. Supported by grant 62FR085A2 of the Délégation Générale à la Recherche Scientifique et Technique.

21 March 1966