present¹⁰. It may be anticipated, however, that some of the mitoses at 72 h have started in daughter cells of those originally present. Long-term cultures may indicate whether these vitamin B₁₂-deficient daughter cells are then able to subdivide further.

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Transfer of Stimulation of Deoxyribonucleic Acid Synthesis in Antigen-stimulated Rabbit Spleen Cell Cultures

LITTLE is known about the nature of the antigenic stimulation to antibody production or the manner in which antigen reaches the necessary site of stimulation. A recent report¹ implicates the sequential action of at least two different types of cells. One type is a mononucleated cell with phagocytic activity, and the other a non-phagocytic cell belonging to the lymphocytic series. Fishman suggests that an RNase sensitive material is transferred from one to the other, and it has been suggested² that complexes between antigenic fragments and RNA may play an important part in antibody formation. A physical means whereby such a transfer might occur^{3,4} has been reported. This communication reports some preliminary experiments which were designed to demonstrate a transfer of antigenic stimulation from one cell culture to another.

It has been shown⁵ that DNA synthesis in spleen cell preparations from previously immunized rabbits is stimulated *in vitro* by the addition of antigen. Furthermore, only brief contact with the antigen is required for stimulation⁶. After washing, less than 0.1 per cent of the added antigen remains. In contrast, 1 per cent of the original amount (or 10 times the amount remaining after washing) of the antigen added to fresh cells is too little to produce stimulation.

This system has been used here to test whether cells 'primed' by brief contact with antigen are able to transfer stimulation to fresh cells from the same spleen. In these experiments, the response of the 'primed' cells was inhibited by irradiation or freezing and thawing. The design of the experiments and results are presented in Table 1.

Table 1. TREATMENT OF CELLS
Spleen cell suspension from immunized rabbits

C	618	
C + C	1,144	(1,236)
CR	71	
C + CR	579	(689)
P	5,168	
P + P	10,600	(10,336)
PR	253	
C + PR	3,200	(871)
PF	1,503	
C + PF	6,143	(2,121)
CR + PR	621	(324)
CR + P	5,087	(5,239)
C + P	7,737	(5,786)

(P) 'Primed' cells. (C) 'Control' cells. (PF) 'Primed' frozen cells.
(PR) 'Primed' irradiated cells. (CR) 'Control' irradiated cells.

Part of the cell suspension (3×10^5 cells) was incubated with 3 mg/ml. of human serum albumin labelled with iodine-131 for 15 min at 37°C. The cells were washed three times in 5 ml. of medium/wash. Determination of residual radioactivity showed that less than 0.2 µg labelled human serum albumin remained/cell culture. Such cells are designated 'primed' cells (P). 'Control' cells (C) were treated identically, except they were not exposed to antigen. One portion of the 'primed' cells received a dose of 300 rads, and these were called 'primed irradiated' cells (PR). 'Control irradiated' cells (CR) were similarly prepared. In addition, another portion of 'primed' cells were frozen and thawed once to give 'primed frozen' cells (PF).

Cultures of such cells were incubated alone or in various combinations, and the rate of DNA synthesis was measured in the second 24-h period, as previously described⁵. The figures represent the total c.p.m. incorporated/culture during 24-h incubation with ¹⁴C-thymidine. The figures in parentheses in Table 1 indicate the expected results of combinations calculated from the figures for single cultures. The results which show evidence of transfer have been underlined.

A definite enhancement of DNA synthesis was observed when 'primed' cells were incubated with control cells. This was clearly shown when DNA synthesis had been inhibited in the 'primed' cells. There was no enhancement of DNA synthesis when 'control' irradiated cells were added to 'control' cells or to 'primed' cells. The enhancement of DNA synthesis obtained when 'primed' cells, which had been frozen and thawed, were added to 'control' cells demonstrated that it is the 'control' recipient cells which respond.

The results of a single experiment are presented, but identical findings were obtained in all of three other experiments using other antigens. The enhancement of DNA synthesis was only observed when the animal had been previously immunized to the antigen. The system is therefore antigen specific.

Although the results are preliminary, two positive statements can be made.

(1) The 'primed' irradiated cells contain some factor capable of stimulating DNA synthesis in control recipient cells.

(2) The presence of this factor is dependent on contact of the 'primed' cells with specific antigen.

The nature of this transfer factor, and the cells concerned, need further investigation before definite conclusions can be drawn. At least three possibilities exist: (A) The minute quantity of antigen taken up represents an extremely potent fraction of the original antigen preparation. (B) The antigen taken up has had its antigenicity enormously enhanced. (C) The cells taking up the antigen hand on some material, other than antigen, which is capable of stimulating cell division. Such a material need not necessarily retain the antigen specificity.

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