Table 3. Local passive transfer of delayed cutaneous hypersensitivity to tumor-specific transplantation antigens measured as the skin reaction at 24 hours. Size of intradermal papules at 5 days is a measure of the ability of the tumor cells in the injected mixtures to grow. Peritoneal exudate (PE) cells were from animals immunized with line 1; r, radius.

Cell mixture (No./10^6) injected in 0.1 ml			Skin reaction at 24 hours ($< r^2 >$, mm^2)	Intradermal papule size at 5 days ($< r^2 >$, mm^2)
PE	Line 7	Line 1		
3	1.5		1.2	5.9
3		1.5	8.5	0
	1.5		0	5.8
		1.5	1.2	7.6

sure growth or suppression of growth (Table 3).

Local passive transfer of delayed hypersensitivity could be accomplished with PE cells from these same animals. Skin reactions caused by mixtures of PE cells with line 1 tumor cells were significantly larger than were reactions caused by mixtures of PE cells with line 7 tumor cells. Reactions of PE cells alone, line 1 alone, line 7 alone, and medium 199 alone had a radius squared less than 1.3 mm^2.

Five days after injection intradermal growth of line 7 tumor cells alone was the same as that of mixtures of PE cells and line 7 tumor cells (Table 3). Peritoneal exudate cells from animals immunized to line 1 caused suppression of growth of line 1 tumor compared to the growth of an equal number of line 1 cells not mixed with PE cells. Additional experiments showed that the growth of tumor cells from either line 1 or line 7 was not suppressed by PE cells from nonimmunized animals.

Tumor-specific transplantation antigens can be detected by these three methods. We do not know, however, whether these three methods detect the same antigens.

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Chloramphenicol: Effects on Mouse Myeloma Cells in Tissue Culture

Abstract. *Within 36 hours of being administered, chloramphenicol (50 micrograms per milliliter) inhibits by 50 percent the rate of protein synthesis in mouse myeloma cells grown in suspension culture. Although there is a decrease in the amount of globulin synthesized, the rate of synthesis per cell is unchanged; the observed decrease is traced to the inhibition of cell proliferation caused by chloramphenicol.*

The cells of the hematopoietic and immune systems are most susceptible to the deleterious effects of chloramphenicol (CM), hematopoietic depression and toxicity being the most common and serious clinical side effects of treatment with this agent (*1*). Chloramphenicol inhibits induced antibody synthesis both in vivo and in vitro (*2*), presumably by inhibiting protein synthesis. However, attempts to demonstrate protein inhibition directly in mammalian systems have led to widely variable results (*3*).

Mouse myeloma cells have been adapted to tissue culture and have been grown continuously in suspension for several years (*4*). These cells offer several advantages. (i) They are susceptible to CM; (ii) they secrete measurable amounts of gamma globulin; and (iii) their growth pattern is highly reproducible. Cells which die remain identifiable and can be counted with no more than 5 percent disintegration loss for 4 to 5 days. This means that cumulative cell counts plotted against time are significant, and rates of division and death per viable cell per hour can be computed from the first derivative of these curves.

Myeloma cells were suspended in fresh growth medium (400×10^3 viable cells per milliliter), and the pooled cells were divided into two equal portions; CM was added to one [50 μg/ml ($1.5 \times 10^{-4} M$)]. Samples (6 ml) were then placed in enough flasks to provide four for each time point (two control, two with CM). Portions (0.5 ml) were then removed in duplicate, and the cells were counted with a Coulter counter to assure that each flask contained the proper number. All flasks were incubated at 37°C. One hour before each interval, four flasks were removed, and 5 μc of C^{14}- labeled amino acids (reconstituted protein hydrolyzate) were added to each. After incubation for 1 hour a portion (0.2 ml) was stained with trypan blue, and the cells were counted in a hemocytometer. Viable cells were defined as those not staining with trypan blue. Duplicate samples (0.5 ml each) were removed, and the cells were counted in the Coulter counter. Total numbers of viable cells were calculated by multiplying the cell number determined with the Coulter counter by the percentages of viable cells as determined by trypan blue exclusion; total numbers of dead cells were determined similarly. Protein synthesis, expressed as disintegrations per minute (dpm), in the fraction precipitable by trichloroacetic acid (TCA) (*5*) and globulin synthesis, measured by the method of radial diffusion (*6*), were determined on the remainder of the culture.

The number of cells in the control cultures increased threefold during 60 hours, whereas in cultures treated with CM the cell number reached a maximum at 36 hours. At this time the treated cultures contained about 60 percent of the number of cells in the control cultures. The total number of dead cells per milliliter was essentially the same in all cultures during 60 hours.

The total number of viable cells can be thought of as the result of cell division and cell death. Because division adds one cell to the total population, the curve of the increase in the total number of cells (viable and dead) as a function of time is also an expression of the total number of divisions. Similarly, a graph of the cumulative increase in the number of dead cells expresses the total number of deaths as a function of time. Comparison of these data shows that the entire difference between the number of viable cells in the control and the treated cultures can be attributed to the far greater number of cell divisions in the former. Cell death contributed nothing, for there was always

a similar number or a greater number of deaths in the control group.

The decrease in the total number of divisions of treated cultures compared to the controls could result from either a decrease in the rate of cell division or from an increase in the rate of cell death in the treated population. If the latter, the decrease in the number of divisions would be the result of fewer viable cells to divide. The rates of cell division and cell death were computed for each time point by dividing the slope of the tangent to the curves at each point by the number of viable cells at that time. The results are expressed as divisions (or deaths) per 10^5 viable cells per hour (Figs. 2 and 3). The decreased number of divisions in the treated cultures is the result of a decrease in the rate of cell division rather than an increase in cell death. Any ambiguity of interpretation is avoided by the fact that treated cells have already almost completely stopped dividing before any significant increase in death rate is seen at 48 hours. This type of analysis shows up an obscured increase in the death rate of treated cells. This may represent a toxic effect of CM which becomes measurable only after 48 hours of treatment. However, even at 72 hours, in spite of a nearly doubled death rate, fewer cells are dying in the treated cultures. This results from previous inhibitions of division, there being less than half as many viable cells as in the untreated population.

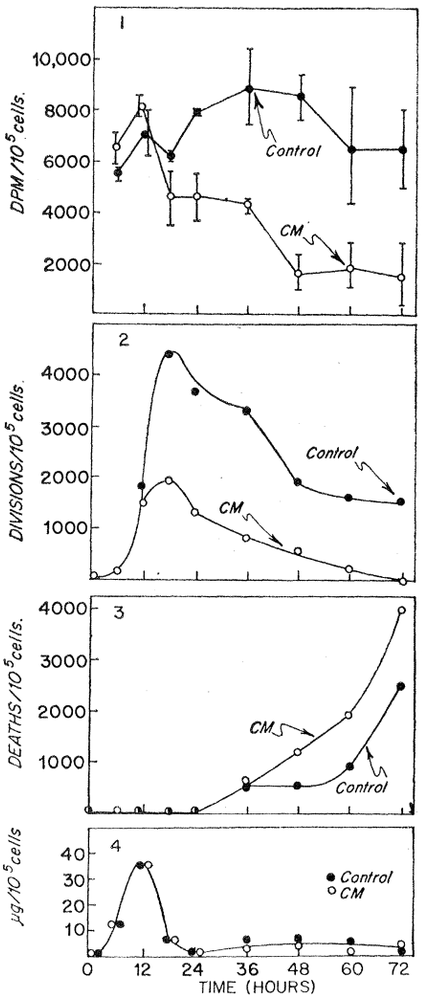

Fig. 1. Rate of protein synthesis. Each point represents incorporation of ^{3}H-leucine (1 μc/ml) into protein of 10^5 viable cells during the preceding 1-hour period. Fig. 2. Rate of cell division (divisions per 10^5 cells per hour). Fig. 3. Rate of cell death (deaths per 10^5 cells per hour). Fig. 4. Globulin synthesized (micrograms of globulin per 10^5 viable cells per hour). Each point represents the amount of globulin present at the particular time. Data represent accumulation of globulin with time.

Rates of protein synthesis (Fig. 1) were measured as radioactivity in TCA precipitates in homogenates of cells which had been incubated with radioactive amino acids for 1 hour. Treatment with CM causes a decrease in the rate of protein synthesis. Furthermore, inhibition of protein synthesis occurs relatively early (between 12 and 24 hours) and is maintained throughout the experiment. Concentrations of CM as low as 10 μg/ml causes significant decrease in protein synthesis in this system (*7*). Globulin synthesis was determined over the 72-hour period. Control cells produced 50 percent more globulin than treated cells, but rates of globulin synthesis per viable cell per hour in control and treated cells are not significantly different (Fig. 4). Thus less globulin is produced in the treated cells because there are fewer cells, rather than because synthesis is inhibited.

By comparing all the rate curves in Figs. 1 to 4 one can understand the time sequence of events. The rate of protein synthesis is first to be affected (Fig. 1), inhibition occurring between 12 and 24 hours, when it approaches 50 percent. Almost simultaneously the rate of cell division decreases. Inhibition of cell division starts at 12 to 18 hours and extends throughout the experiment (Fig. 2). The apparent decrease in the degree of inhibition at later time is due to a decrease in the rate of division of control cells, resulting from unfavorable environmental conditions. During the phase of inhibition of protein synthesis and of cell division, there is no increase in death rate. Thus the protein inhibited at this time, although necessary for division, apparently is not necessary for viability.

The decrease in globulin synthesis in this system is analogous to the decrease in antibody production observed by others (*2*). However, this effect results from a decrease in the rate of cell division by the myeloma cells. In antibody induction experiments where cellular proliferation is of primary importance, one might expect an even greater effect on total globulin synthesis than observed in our experiments. In this system a major effect of the observed decrease in protein synthesis is a decrease in cell proliferation. This correlates well with the observation that CM administered before and during antigenic stimulation almost completely inhibited specific antibody synthesis (*2*). However, when treatment is delayed until after the inductive (proliferative) phase of a secondary response, inhibition of antibody synthesis is markedly reduced.

These data demonstrating the inhibition of mammalian cell proliferation by chloramphenicol could also explain the sensitivity of proliferative phases of erythropoiesis to this drug in patients treated for anemia (*1*). Finally, the demonstration that CM can affect protein synthesis, and the rate of division in these cells before causing an increased death rate, provides a valuable tool for additional studies of protein synthesis in this mammalian system.

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