glutinating antibodies by the polyvinylprolidone technique (*5*). The heat eluate showed a specific hemagglutinin titer of 2^6 to 2^7 against A/Jax red blood cells. Specific hemagglutinins were not detected in the eluate from control nonimmune cells or in the final washing fluid of immune cells or in membrane-free extracts of immune cells. Although the hemagglutinin was destroyed by exposure to 0.1*M* 2-mercaptoethanol for 60 minutes at 37°C, trials to establish its sedimentation behavior by sucrose-gradient techniques were not satisfactory.

The eluates were tested further by Terasaki's cytotoxicity test (*6*). Five different heat eluates from immune cells were capable of activating complement, as shown by their capacity to kill approximately 70 percent of normal A/Jax lymph-node cells in the presence of fresh rabbit serum.

The influence of metabolic inhibitors on the adherence and plaquing capacity of immune cells was tested. A suspension (0.5 ml) containing 2×10^6 of well-washed immune cells per milliliter was incubated for 2 hours at 37°C in 5.0 ml of Eagle's minimal essential medium plus high concentrations of cell inhibitors as follows: 0.1*M* sodium azide, 0.004*M* 2,4-dinitrophenol, 0.05*M* sodium fluoride, 10.0 μM actinomycin D, 100 μg of chloramphenicol per milliliter, and 200 μg of puromycin per milliliter. The cells were chilled to 4°C, sedimented, and suspended in 0.2 ml of Eagle's minimal essential medium. One drop of each treated cell suspension was then placed on a monolayer of A/Jax fibroblasts and a monolayer of L-cells. After 5 minutes, the monolayers were washed vigorously three times with a total of 30 ml of physiologic saline and stained by the plaque method (Fig. 2). Untreated control cells and inhibitor-treated cells adhered equally well to target cells. Specific adherence of the immune cell to the target cell apparently does not require a metabolically active immune cell. Actinomycin D and chloramphenicol-treated cells were washed before the plaque test. In some cases chloramphenicol was added to the medium (Fig. 3). Both actinomycin D and chloramphenicol blocked plaque formation. However, chloramphenicol-treated cells only remained inactive when initial treatment was supplemented by maintaining a concentration of 100 μg of chloramphenicol per milliliter of culture medium (Fig. 3, *A* and *B*). Whereas control immune peritoneal cells produced plaques (Fig. 3*D*), control starch-induced nonimmune C57B1/Ks peritoneal cells at the high dose of 2×10^6 cells failed to produce plaques (Fig. 3*C*).

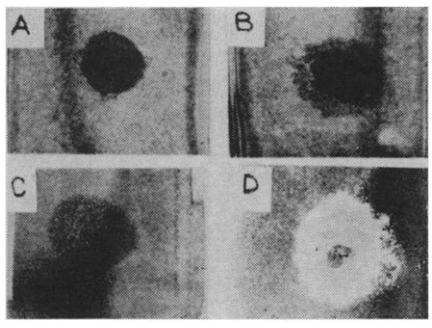

Fig. 3. Effect of chloramphenicol and actinomycin-D treatment on the capacity of immune C57B1/Ks macrophages to cause destruction of L-cell target monolayers after 48 hours. (*A*) Chloramphenicol-treated cells. (*B*) Actinomycin D–treated cells. (*C*) Normal peritoneal untreated cells. (*D*) Untreated immune cells (the central dark spot is a wax pencil mark).

With respect to the chance that complement or its components may participate in plaque formation, three possible sources of complement were considered: (i) "free" humoral complement, (ii) humoral complement which may become fixed to immune cells in vivo, and (iii) complement formed by either the immune cell or the target cell. The participation of free humoral complement appears to be ruled out by our observations that well-washed immune cells exhibit full plaquing activity in medium without serum or in medium containing heat-inactivated calf serum. The second possibility, that absorbed complement is present on the surface of the immune cell, was tested. Immune macrophages were unaffected by treatment with heat-inactivated rabbit antiserum to mouse tissue. No evidence was obtained that complement participates in plaque formation.

Thus, immune peritoneal macrophages derived from C57B1/Ks mice, immunized against A/Jax antigens, possess membrane-associated antibodies that may be responsible for specific contact adherence of the immune cell to the target cell. That specific adherence was not blocked by a variety of cell-inhibiting agents is consistent with the concept that adherence is passive and results solely from the reaction of a surface membrane antigen of the target cell with surface membrane-bound antibody of the immune cell.

The additional observation that actinomycin D– or chloramphenicol-treated immune cells were unable to destroy the target cells even though they adhered suggests that to form plaques the immune cell must have biosynthetic activity. In contrast some trials indicate that treatment of target cells with metabolic inhibitors does not affect plaque formation.

GALE A. GRANGER
RUSSELL S. WEISER
Department of Microbiology, University of Washington, School of Medicine, Seattle

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Sulfur Mustard: Reaction with L-Cells Treated with 5-Fluorodeoxyuridine

Abstract. *After exposure to 5-fluorodeoxyuridine, L-cells are considerably more sensitive to the lethal effect of sulfur mustard than after they have been released from this block by addition of thymidine and allowed to proceed into the G2 phase of the division cycle. Nevertheless, for both populations, the amounts of mustard bound per cell and per nucleus (expressed as the amount of mustard per unit of protein) were the same. Likewise, the amounts of mustard bound per unit of DNA were the same for both populations.*

Cultured L-strain mouse cells were more sensitive to the lethal effect of nitrogen and sulfur mustards when the cells were synthesizing DNA than after they had entered the G2 period (*1*). This result was obtained with cell populations that had been treated with 5-fluorodeoxyuridine (FUDR) and then with thymidine, which releases the cells from the inhibitory effect of the FUDR. The cells then move in a wave successively through phases S and G2. Beyond this point the synchrony diminishes rapidly. Since the

time of the earlier report (*1*), some of the characteristics of the L-cell line have changed. The doubling time has decreased from 20 hours to 16 hours and the sensitivity to sulfur mustard has increased significantly. I have reinvestigated the sensitivity to sulfur mustard of cells in the S and G2 phases and report some chemical data concerning the reactivity of the cell compounds with mustard.

The subline of L-strain mouse cells was maintained in continuous suspension culture at 37°C in medium CRML-1066, containing 5 percent horse serum, penicillin, and streptomycin but with thymidine excluded (*1*). During exponential growth the doubling time of these cells was 16 hours. The number of cells in suspension was determined by means of a Coulter electronic cell counter. The S-phase cells were produced by exposing an exponentially growing suspension culture, containing 1 to 2 $\times$ 10^5 cells per milliliter, to $10^{-7}M$ FUDR for 14 hours (*2*); 4½ hours after the addition of thymidine to such a culture (final concentration, 10 μg/ml), the cells were in the G2 phase (*3*).

Survival curves were obtained by examination of samples from the culture of S or G2 cells. After the appropriate dilution the cells were added to petri dishes containing the plating medium, which consisted of CRML-1066 with antibiotics, thymidine (10 μg/ml), 5 percent horse serum, and 5 percent fetal calf serum. Sulfur mustard in methanol, freshly prepared from a stock solution, was then added to the dishes. The dishes were incubated for 10 days at 37°C in a humidified atmosphere containing 5 percent CO_2. The colonies were then stained with 0.5 percent aqueous methylene blue and counted under a low-power microscope. A minimum of 25 cells constituted a colony. The stock solution of mustard, 5 mg per milliliter of methanol, was kept satisfactorily at −20°C. The amount of methanol in all dishes including the controls was in the proportion of 0.05 ml per 5 ml of medium, and the plating efficiency of the controls was usually 80 percent or higher. After mustard treatment cells that were not able to divide and form colonies persisted in the form of "giant" (*4*) or "feeder" cells, and their numbers influenced the numbers of colonies that developed. Therefore, sufficient extra "feeder" cells were added to each dish to give a total of 10^5 per dish. The extra "feeder" cells were prepared by treating a small suspension culture with 2 μg of mustard per milliliter of culture for 1 hour at 37°C.

Sulfur mustard labeled with S^{35} (initial specific activity, 30 mc/mmole) was obtained from the Radiochemical Centre, Amersham, England. The preparation was dissolved in methanol and stored at −20°C. It was diluted further with methanol and added to suspension cultures of S or G2 cells to give a final concentration of 0.6 μg/ml. After treatment for 1 hour at 37°C, samples of the culture were centrifuged, and the collected cells were washed twice by suspension in phosphate-buffered saline (*5*) and centrifuged again. The radioactivity in the whole cells was measured by suspending the cells in phosphate-buffered saline and catching a sample on a Millipore filter (pore size, 0.45 μ). The filters were placed in toluene-phosphor solution and counted in a liquid scintillation counter (Nuclear Chicago). Nuclei were prepared (*6*), and samples of these were collected on Millipore filters; their radioactivity was measured as described for whole cells. DNA was isolated from whole cells by the method of Djordjevic and Szybalski (*7*). An important point concerning the use of this method in my study is that the yields of DNA are nearly quantitative, the DNA is free of RNA, and any protein present seems to have come from the ribonuclease used in the preparation. The DNA was precipitated from solution by addition of an equal volume of 1*N* perchloric acid; the precipitate was either collected on a Millipore filter and counted as described, or it was dissolved in toluene-phosphor solution with the aid of hyamine and counted. Protein was measured by the method of Oyama and Eagle (*8*), and DNA was measured by Burton's method (*9*).

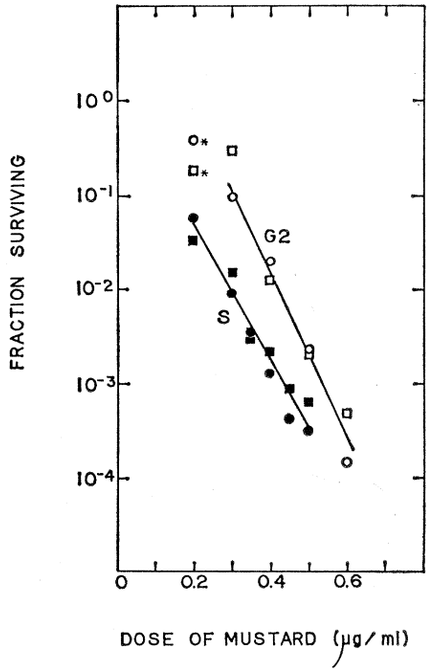

Fig. 1. Survival of reproductive capacity of S and G2 populations of L-cells after treatment with sulfur mustard. The lines are computed regression lines. The two points marked with asterisks were not included in the calculation of the upper regression line because they lie in the shoulder region of the survival curve.

Figure 1 shows the survival for S and G2 populations. The lines were derived by regression analysis and their slopes are not significantly different (*10*). This result differs from that obtained previously, in which the slope of the survival curve for S-phase cells was greater than that for G2-phase cells (*1*). In the previous study the degree of cell killing by the doses of mustard used was extended about two logarithmic units for the G2-phase cells. Further, the survival curve for these cells had a broad shoulder. It now seems possible that a straight line was fitted to points that lay in the shoulder region, so that the resulting slope was erroneously shallow. My present data do not suffer from this defect.

Because the slopes of the two survival curves are not different it may be concluded that the target components of the cells in both S and G2 populations are being inactivated at the same rate. This contention receives support from the data (Table 1) which show the amount of sulfur mustard that has combined with the whole cells, nuclei, and DNA from populations of S and G2 cells. After thymidine has been added to the FUDR-blocked cultures and during the subsequent 4.5-hour interval, which takes the cells into the G2 period, there is no increase in cell number. However, the cells increase their protein and RNA content, and they synthesize enough DNA to generate the pre-mitotic amount of this material. When equal volumes of culture are analyzed, whole cells, nuclei, and DNA from G2 populations contain more radioactive mustard than their counterparts from S populations. However, when radioac-

Table 1. Radioactive mustard associated with whole cells, nuclei, and DNA after reacting S- and G2-phase cells with mustard (final concentration 0.6 μg/ml) for 1 hour at 37°C. The radioactivity of all the samples, except the DNA preparations in experiment III, was measured by the liquid scintillation technique (the samples having been collected on Millipore filters). The filters were placed in toluene-phosphor solution and counted. The DNA samples in experiment III were dissolved in toluene-phosphor solution and counted. All values are the average of two estimations.

Experiment	Specific activity (count min^{-1} μg^{-1})		
	Whole cells*	Nuclei*	DNA†
	S phase		
I	0.555	0.511	
II	.541	.533	
III			12.0
IV			8.47
	G2 phase		
I	0.523	0.452	
II	.519	.531	
III			11.4
IV			8.70

* Per microgram of protein. † Per microgram of DNA phosphorus.

tivity values for whole cell and nucleus are expressed per unit of protein and those for DNA are expressed per unit of DNA (Table 1) there is no difference between the two populations.

It seems likely that the DNA in S-phase cells and the DNA in G2-phase cells have different molecular configurations and perhaps different degrees of association with histones and other nuclear proteins. Therefore it is noteworthy that the extent of mustard reaction with DNA is the same in S and G2 cells.

According to target theory, if the number of targets in the unit to be destroyed is increased, the targets behave independently with respect to the inactivating agent (*11*). The G2 cells contain almost twice as much DNA as do the S cells under the present experimental conditions. If DNA is the target component of the cells, and G2-cell and S-cell DNA do not react differently from each other with mustard one would expect the following: the linear portion of the G2-cell survival curve would be parallel to that of the S cells but would be displaced above it by a factor of almost 2. That is, the extrapolation number of the G2 curve would be twice that of the S curve. In fact the curves are displaced by a factor of about 10. This could be taken to mean that another target is involved. It could also mean that some kind of repair mechanism is operative and that G2 cells can take advantage of this mechanism.

In order to obtain some idea of the quantitative nature of the lethal effect of sulfur mustard on L-cells, the following calculations were made. From the data of experiment III (Table 1) where a reliable estimate of the counting efficiency was made it was calculated that there was one alkylation per 48,000 nucleotides in DNA. From Fig. 1, the D_0 value (*12*) or dose which on the average will inactivate a cell is 0.06 μg/ml. Since alkylation of the cellular DNA is directly proportional to the dose of mustard (*13*), the D_0 would yield therefore one alkylation per 480,000 nucleotides. If the average gene contains about 3000 nucleotides (*14*) then about one gene in every 160 (480,000/3000) could be alkylated. Some genes undoubtedly would escape alkylation, while others would suffer several alkylations. If gene inactivation is the critical action of mustard in producing cell death, then the foregoing calculations suggest the feasibility of this process. At the same time the calculations indicate the small extent of gene damage that will produce cell death. Such a consideration is even more marked if only about one in five alkylations or perhaps fewer yield cross-links in the DNA and it appears that the latter is the inactivating reaction (Brookes and Lawley, *13*).

I. G. WALKER
Cancer Research Laboratory,
University of Western Ontario, London

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15. I thank Mrs. Patricia Whiteside, H. Chew, and Mrs. Jean Smith for technical assistance. Supported by a grant from the National Cancer Institute of Canada.

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Pesticide Residues in Total-Diet Samples

Abstract. *Small amounts of pesticide residues were found in food samples from 18 markets consisting of 82 foods collected from three different geographical areas. The samples were separated into twelve similar classes of foods, made ready to eat, and analyzed by methods capable of detecting small quantities of 50 common pesticide chemicals.*

Residues of pesticides in crops that are shipped are subject to government limitations, but obviously the real significance of the residues lies in the amount actually ingested by animals and humans. Data on the actual amounts of the residues in food as shipped do not give a clue to the amount that will be ingested. There is, therefore, a continuing need for this information specifically.

Limitations in the form of legal and safe tolerances have been established by the Food and Drug Administration for raw agricultural products as shipped in interstate commerce. Some states have established similar tolerances for intrastate control. The 1954 statement of the National Academy of Sciences with its subsequent revisions (*1*) is used as a basic guide. The report issued by the President's Science Advisory Committee discusses in some detail the mechanism and criteria used in setting tolerances (*2*).

We planned our investigation to provide additional information and expand the data furnished by experiments on specific food items and on specific types of food processing.