glycerophosphate dehydrogenase activity in heart, coupled with the veritable absence of α-glycerophosphate oxidase from mitochondria of this tissue (*9*), suggests that the α-glycerophosphate cycle is of little significance in heart.

Malic dehydrogenase activity was remarkably high, especially in heart, kidney, diaphragm, and liver. In fact, malic dehydrogenase was more active than lactic dehydrogenase in all tissues except leg muscle and testis. The present values for malic dehydrogenase were two- to four-fold those reported for several tissues of other species (*6, 8*).

The mean values of 330, 30, and 150 μmole of DPNH oxidized per minute per gram of wet weight of skeletal muscle with the lactic, the α-glycerophosphate, and the malic dehydrogenases, respectively, were in marked contrast to titers of 0, 1230, and 400 for these same three enzymes in flight muscle of flies (*5*). Enzymatic activities of extramitochondrial preparations of the various tissues were not appreciably different from those of homogenates.

Although measurements of the optimum activities of the three dehydrogenases give estimates only of the potential of each pathway, a comparison of the values in the different tissues suggests that each tissue has a unique pattern for oxidation of extramitochondrial DPNH. Additional evaluation of the pathways was obtained by determining the concentrations of the redox metabolites in tissues at "rest" and while performing work. As an example, concentrations in skeletal muscle of α-glycerophosphate, malate, and lactate increased in a characteristic fashion with repeated isometric contractions (*10*). Other changes in concentration of metabolites during physiological activities of tissues are described elsewhere (*11*).

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Inhibition of Antibody Plaque Formation by Sensitized Lymphoid Cells: Rapid Indicator of Transplantation Immunity

Abstract. *Spleen cell suspensions obtained from mice immunized with sheep erythrocytes form localized zones of hemolysis ("antibody plaques") when incubated in agar gel containing sheep red blood cells and complement. Plaque formation can be inhibited by prior incubation in vitro with spleen cell suspensions from another strain of mice previously sensitized to the first by spleen cell transplant. Suppression of plaque formation was found to be quantitatively related to the number of cells incubated and apparently reflected a homograft reaction in vitro of one spleen cell population against another. Plaque inhibition may be a useful indicator of transplantation immunity.*

Immunologic incompatibility between unrelated individuals within a species is often evaluated by determining the time required for overt rejection of grafted tissues (*1*). There have been several attempts to develop rapid and reproducible procedures for demonstrating either transplantation immunity or histocompatibility between graft donors and recipients. For example, "tissue typing" procedures in vivo involving intradermal injection of peripheral recipient leukocytes into prospective donors have been reported as relatively rapid and reliable indicators of tissue incompatibility (*2*). Similarly, a "tissue culture" method in vivo for assaying homograft reactions has been described by Celada and Carter (*3*). In their procedure, immunologically inert x-irradiated recipient mice were injected with lymphoid cell suspensions from two strains of mice, one of which had been previously immunized with sheep erythrocytes. Tissue incompatibility between the two strains was assessed by measuring the degree of suppression of expected agglutinin formation in the irradiated recipients several days after cell transfer.

However, a test for histocompatibility ideally should be performed completely in vitro within a short period of time. In this regard, the use of cytotoxic or agglutinating antiserums for "typing" in vitro of peripheral leukocytes has been reported as a possible method for detecting antigenic differences among individuals (*4*). Similarly, interaction in vitro of lymphoid cells from unrelated individuals in tissue culture media has been recently presented as a possible method for demonstrating homograft reactivity (*5*). In this procedure, incubation of peripheral leukocytes from two unrelated individuals in tissue culture medium resulted in a specific increase in the number of mitotic figures and large lymphoid cells during a period of about a week. No increased mitotic activity was observed after incubation of lymphoid cells from genetically identical individuals.

This report is concerned with detection of homograft sensitivity in mice by an adaptation of the recently described localized hemolysis techniques (*6, 7*). In the method developed by Jerne *et al.* (*6*), immunologically competent lymphoid cells from animals sensitized with foreign erythrocytes form localized zones of hemolysis when incubated at 37°C with erythrocytes suspended in solidified agar gel, and then incubated with guinea pig complement. In the procedure described independently by Ingraham and Bussard (*7*), culture medium is thickened with high-viscosity cellulose gum (carboxymethoxycellulose) instead of agar. Lymphoid cells from erythrocyte-immunized animals are suspended in the thickened medium, to which washed erythrocytes and guinea pig complement are added. This mixture is incubated directly on microscope slides. Small zones of hemolysis, presumably surrounding antibody-secreting cells, develop during incubation. These localized hemolysis procedures permit rapid scoring of the number of individual antibody-releasing cells present in a given population of lymphoid cells. The use of Jerne's antibody-plaque method in agar gel as a possible indicator in vitro of homograft reactivity between lymphoid cells from two strains of mice is presented here.

For these experiments, "target" cells

Table 1. The number of hemolytic plaques formed per milliliter of cell suspension after incubation of immune "target" spleen cells from NIH mice and sensitized spleen cells from C_3H mice sensitized to NIH mouse cells or control preparations. One milliliter of spleen cell suspension from immune NIH mice was incubated with 1.0 ml sensitized C_3H spleen cell suspension or controls at 37°C for 2 hours. Then 0.1 ml of incubation mixture was plated with 0.1 ml of washed sheep red blood cells (10 percent) in 2.0 ml of melted 0.7 percent agar containing diethylaminoethyl dextran.

NIH "target" spleen cells (No./ml)	C_3H "killer" spleen cells (No./ml)			Other cells (No./ml)			Hanks solution
	6.8×10^6	8.3×10^5	1.6×10^5	Heated "killer" C_3H cells, 5.3×10^6	Normal C_3H cells, 6.1×10^6	Normal NIH cells, 4.5×10^6	
3.5×10^6	126	368	516	893	820	873	865
5.4×10^5	37	56	78	110	97	120	132
4.8×10^4	2	4	8	11	15	28	12

were obtained from spleens of 8- to 10-week-old NIH (strain A albino) mice which had been primed 4 days previously with a single intraperitoneal inoculation of washed sheep erythrocytes (S-RBC). Spleen cell suspensions obtained from such immunized NIH mice produced high concentrations of agglutinins and hemolysins to sheep red cells when incubated in tissue culture medium at 37°C for periods of 1 to 3 weeks. Cells from nonimmunized animals did not produce readily detectable hemolysins. Suspensions of immune cells formed relatively large numbers of antibody plaques, as described below, when incubated in semisolid agar containing sheep erythrocytes and complement. "Killer" spleen cell suspensions were obtained from 8- to 10-week-old C_3H mice which had been injected intraperitoneally 10 to 14 days previously with 10 to 20 million nucleated spleen cells from normal NIH mice. Similar results to those reported here were obtained when C_3H mice were sensitized to tissue antigens of NIH mice by one or more skin homografts from normal NIH donors. Sensitized C_3H "killer" spleen cells were incubated in vitro with spleen cell suspensions from the immune NIH mice in order to test their influence on antibody plaque formation.

The assay for plaque formation was performed essentially as described by Jerne (*6*). In brief, the method used was as follows: 0.1 ml of immune spleen cell suspension from NIH mice or incubation mixture was rapidly added to 2.0 ml of 0.7 percent melted Noble agar in buffered Hanks solution, *p*H 7.2, containing 1 mg DEAE-dextran (2×10^6 M.W.) and maintained at 48° to 50°C. To this mixture, 0.1 ml of 10 percent fresh washed sheep erythrocytes in Hanks solution was rapidly added. After thorough but gentle mixing, the warm cell-agar suspension was overlaid on a 3-mm thick base layer of solidified 1.4 percent agar prepared in 100-mm diameter plastic petri dishes. After solidification of the upper layer at room temperature, the plates were incubated for 1 hour at 37°C, then treated with 5 ml of guinea pig complement (diluted 1:10) and reincubated for an additional 30 minutes at 37°C. Antibody plaques could be readily visualized as clear zones of hemolysis against a pink background of unlysed red cells. The plaques were stabilized, and the plates could be stored in the cold for extended periods of time by staining with freshly prepared benzidine-acetic acid-H_2O_2 solution (*6*). The number of plaques per plate was recorded with a Quebec bacteria colony counter. Petri dishes containing a dilution of cells which resulted in 50 to 300 plaques per plate were used for determination of the number of plaque-forming cells per total number of viable lymphoid cells plated. Each dilution—and cell-suspension mixture—was plated in triplicate, and the average count per number of viable nucleated cells plated was used for calculations.

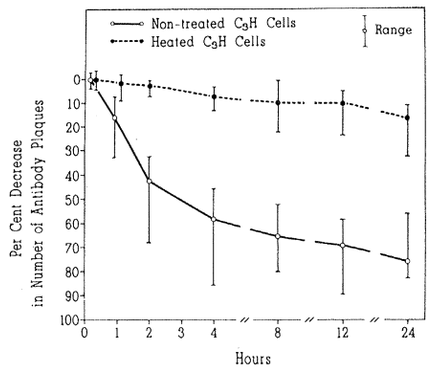

Fig. 1. Relative decrease with time of antibody plaque formation by "target" spleen cells from immune NIH mice (2×10^6 cell/ml) incubated with equal numbers of either untreated (open circles) or heated "killer" C_3H spleen cells (closed circles) compared to plaque formation by immune NIH cells incubated with Hanks solution only. Each point represents mean plaque counts of at least six separate incubation mixtures. Plaque counts were recorded as average number of plaques per 10^6 viable nucleated cells remaining at each time period.

The optimum time for maximum antibody plaque formation by spleen cells from S-RBC immunized NIH mice was found to be 4 to 5 days after a single injection of erythrocytes. For a typical homograft-plaque inhibition experiment, a dozen NIH mice were injected intraperitoneally with 0.5 ml of 20 percent washed sheep erythrocytes. Four days later, blood specimens were obtained by retro-orbital puncture to determine the level of circulating anti-sheep hemolysins. After killing those mice whose blood showed high concentrations of hemolysins, spleens were obtained, and cell suspensions were prepared by "teasing" the spleens into sterile Hanks solution containing penicillin and streptomycin (*8*). The cells were filtered through sterile gauze, washed three times in Hanks solution by serial centrifugation in the cold, and resuspended at concentrations varying from 10×10^6 to 5×10^4 viable nucleated cells per ml. Viability was estimated by trypan blue dye exclusion tests, and total cell counts were determined with a hemocytometer. To 1.0 ml of these "target" cell suspensions in stoppered roller tubes was added 1.0 ml of either (i) suspensions of viable "killer" C_3H spleen cells obtained from the C_3H mice sensitized to NIH tissue antigens; (ii) heat treated (56°C for 30 minutes) sensitized C_3H cells; (iii) control nonsensitized C_3H spleen cells; or (iv) sterile Hanks solution or normal untreated NIH spleen cells. The tubes were incubated at 37°C. At various times thereafter, samples were removed aseptically and washed several times by serial centrifugation with Hanks solution, and agar pour plates were made to determine the number of antibody plaque forming cells per million viable nucleated cells.

Figure 1 indicates results observed in a typical series of experiments in which equal numbers of spleen cell suspensions from C_3H mice sensitized to NIH cells were incubated with spleen cell suspensions from the NIH mice immunized against S-RBC for periods up to 24 hours. Total viability of the cells remained relatively stable during the first hours of incubation. Approxi-

mately 20 to 30 percent of the nucleated cells were nonviable at 6 to 8 hours, while more than half of the cells were nonviable at 24 hours. At the same time, there was a marked reduction in the number of antibody plaques formed. As an example, after 2 hours of incubation there was a 35 to 65 percent reduction in the number of antibody plaques per total number of viable lymphoid cells compared to control incubation mixtures containing immune cells from NIH mice and Hanks solution only. After 8 hours there was a 50 to 80 percent decrease in plaque count. In contrast, there was no significant decrease in plaque counts per number of viable cells when spleen cells from immunized NIH mice were incubated with nonsensitized, normal C$_3$H cells, or heated, nonviable "killer" C$_3$H cells (Fig. 1 and Table 1). Similarly, there was no demonstrable decrease in plaque formation after incubation of immune NIH cells with normal, isologous NIH cells for varying periods of time from 2 to 24 hours. In several instances there was an indication of increased antibody plaque formation at 24 hours or longer when immune NIH cells were incubated with normal NIH cells instead of Hanks solution.

Suppression of plaque formation was closely related to the relative concentration of immune NIH and "killer" C$_3$H cells in the incubation suspensions. An initial ratio of two C$_3$H "killer" cells for each NIH "target" cell resulted in a 60 to 80 percent reduction in the relative number of plaques formed after 2 hours incubation (Table 1). A lower ratio resulted in less suppression. One sensitized C$_3$H cell for each ten "target" cells resulted in only slight inhibition of plaque formation.

The results indicate that the antibody plaque forming capability of immune lymphoid cells may be inhibited by prior incubation in vitro with lymphoid cells from homograft sensitized mice. Significant inhibition may be detected after only a few hours incubation of the two sets of cells. Such inhibition apparently reveals tissue incompatibility between "target" and "killer" strains. Inhibition does not occur if "killer" cells have been rendered nonviable by heating. Other experiments indicate that serum samples from homograft sensitized "killer" mice are much less effective inhibitors of plaque formation than lymphoid cells (*9*). However, serum specimens from rabbits hyperimmunized either with mouse γ-globulin (*7S* or *19S*) or with mouse spleen cell suspensions are markedly effective in preventing plaque formation when incorporated into agar together with plaque-forming cells and sheep RBC (*9*). These observations and those reported here lend support to the belief that the Jerne technique detects new antibody formation rather than the release of preformed antibody.

The experiments have been limited to spleen cells from "target" mice primed only with red blood cell antigens. Attempts to couple protein antigens, such as bovine serum albumin or egg albumin, to red cells by tannic acid or bisdiazotized benzidine methods have not been successful in our laboratories as far as the antibody plaque technique is concerned. However, preliminary experiments in which red cells have been coated with polysaccharide antigens derived from Gram-negative bacteria (*Shigella* microorganisms) have resulted in plaque formation with spleen, lymph node, and peripheral leukocytes from immunized mice (*9*). This method should be of use in studies concerned with suppression of plaque formation with peripheral leukocytes obtained from mice immunized with bacteria and plated with red cells coated with bacterial antigen.

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Physiological Studies on Fruit Development by Means of Ovule Transplantation in vivo

Abstract. *Young fertilized ovules belonging to different species, genera, and families were transplanted onto pepper placenta and grown in vivo to mature, viable seeds. This suggests that the physiological requirements for growth of these ovules may be similar between plant species that are quite different taxonomically.*

The principal factors that control fruit growth are not understood. An extensive search is in progress (*1*, *2*) to determine whether each species or genus may require specific growth factors for the development of its own fruit. We have performed transplantation experiments with young ovules in vivo which suggest that the chemical control of the developing fruits may be similar for some varieties, species, genera, and families.

Explants consisting of young fertilized ovules (hereinafter referred to as ovules) of several types of fruits were inserted in the placenta of growing, attached fruits of pepper (*Capsicum frutescens* L. var. California Wonder). The morphology of the pepper fruit makes it particularly suitable for this technique (*3*).

A small triangular opening, each side measuring 1 cm, was made in the pericarp of the pepper (Fig. 1). Through this opening the operation was performed on the pendant placenta by means of surgical instruments. To

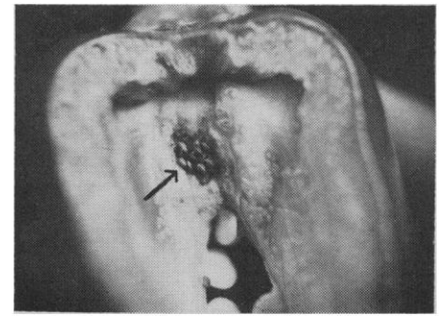

Fig. 1. Pepper fruit attached to parent plant showing the window (cut larger for photograph) made in the pericarp wall. Transplanted strawberry receptacle with achenes can be seen nearing maturation (arrow).