The observed patterns are not very dissimilar from the patterns predicted by this assumption. When the assay for ADH can be performed quantitatively on the gel this hypothesis can be tested directly. Experiments are under way to distinguish between the two hypotheses.

In $Adh^F/Adh^S$ heterozygotes, the parental and intermediate bands form an interdigitating trio of patterns. Bands 1, 2, and 3 form the primary group and contain most of the ADH activity; bands 4, 5, and 6 form the secondary group and comprise a minor portion of the activity; bands 7, 8, and 9 form another group and contain a very small percentage of the total alcohol dehydrogenase of the fly. Bands 2, 5, and 8 are present only in the heterozygote, indicating that *Drosophila* alcohol dehydrogenase contains two subunits. Thus it is apparent that these hybrid bands may be formed by the association of one slow and one fast subunit, as postulated for hybrid bands of maize esterases by Schwartz (*7*). Johnson and Denniston (*1*) reported that alcohol dehydrogenase isozymes exist in *Drosophila* in "strong zones" and "weak zones," each consisting of a three-banded pattern in the hybrid condition. These correspond to the primary and secondary groups described above. They also postulate a dimer structure for the alcohol dehydrogenases of *Drosophila* to explain their observations.

E. H. GRELL
K. BRUCE JACOBSON
J. B. MURPHY

*Biology Division,*
*Oak Ridge National Laboratory,*
*Oak Ridge, Tennessee*

### References and Notes

1. F. M. Johnson and C. Denniston, *Nature* **204**, 906 (1964).
2. T. R. F. Wright, *Am. Zool.* **1**, 476 (1961); J. L. Hubby, *Genetics* **47**, 961 (1962); H. Kikkawa and Z. Ogita, *Jap. J. Genet.* **37**, 394 (1962); E. M. Pantelouris and E. J. Duke, *Genet. Res.* **4**, 441 (1963); W. J. Young, J. E. Porter, B. Childs, *Science* **143**, 140 (1964); L. Beckman and F. M. Johnson, *Genetics* **49**, 829 (1964); L. Beckman and F. M. Johnson, *Hereditas* **51**, 212 (1964).
3. S. Raymond and Yi-Ju Wang, *Anal. Biochem.* **1**, 391 (1960).
4. M. M. Dewey and J. L. Conklin, *Proc. Soc. Exptl. Biol. Med.* **105**, 492 (1960).
5. C. B. Bridges and K. S. Brehme, *The Mutants* of Drosophila melanogaster (Carnegie Inst. Wash. Publ. No. 552, 1944).
6. L. A. Costello and N. O. Kaplan, *Biochim. Biophys. Acta* **73**, 658 (1963).
7. D. Schwartz, *Proc. Natl. Acad. Sci. U.S.* **52**, 222 (1964).
8. Research sponsored by the U.S. Atomic Energy Commission under contract with the Union Carbide Corporation. We thank D. L. Lindsley, Jr., for constructive discussion of one of the hypotheses proposed.

14 May 1965

## Abrogation of Allogeneic Inhibition by Cortisone

Abstract. *Tumor cells from homozygous mice grow better upon transplantation to syngeneic mice than to $F_1$ hybrids between the tumor strain and a foreign strain. The inhibition of cell growth in the hybrids (called allogeneic inhibition), which is detected by tumor transplantation into mice, could be abrogated by treatment of the recipient mice with cortisone acetate. Cortisone also abolished allogeneic inhibition in vitro; abolition was detected by treating tumor cultures with cell extracts containing foreign isoantigens of the H-2 type.*

Tumor cells from homozygous mice regularly grow better upon transplantation to syngeneic mice than to various allogeneic hosts, including $F_1$ hybrids between the tumor strain and a foreign strain, the homozygous mice developing tumors in a higher frequency and after a shorter latency period (*1, 2*). This phenomenon has been termed syngeneic preference or allogeneic inhibition, the latter term referring to the deficient growth in the $F_1$ hybrids (*3*).

The mechanism of allogeneic inhibition was studied by explanting tumors of C57BL and A/Sn × A.CA $F_1$ hybrid origin in culture and exposing the cultures either to a cell-free homogenate or to an antigenic extract, which was prepared from A/Sn or C57BL mice and which contained either foreign or matching histocompatibility antigens of the H-2 type. The experiments showed that homogenates and extracts containing antigens which were mismatched possessed a specific cytotoxic or growth-inhibitory effect when compared to similar but matching preparations, an indication that allogeneic inhibition, as detected by tumor transplantation to mice, probably depends on exposure of the grafted cells to foreign histocompatibility antigens of the hosts (*4*).

An incidental finding was that cortisone decreased allogeneic inhibition, as detected by tumor transplantation to syngeneic and $F_1$ hybrid animals. Subsequent related experiments have shown that allogeneic inhibition in tissue culture could also be abolished by cortisone. An account of these results is given here.

Two tumors were used for the experiments in vivo, MC57S and MLG; both were fibrosarcomas originally induced by methylcholanthrene in strains C57BL and C57L. They were kept by serial transplantation in syngeneic animals for 27 and 23 passages, respectively, prior to the present tests. For each experiment, syngeneic animals were used as well as $F_1$ hybrids between the tumor strain and a foreign strain (C3H × C57BL $F_1$ and A × C57L $F_1$, respectively). Female mice, aged 1 to 2 months, were used. Mice of each type were divided into two similar groups, one being an untreated control and the other being given one intramuscular injection of 2.5 mg cortisone acetate (Upjohn) 2 days before tumor inoculation. Suspensions of tumor cells were prepared by treating the tumors with trypsin; $10^4$ of the trypsin-treated cells were injected subcutaneously in the backs of the mice. The mice were inspected every 3rd to 5th day after inoculation.

Table 1. Effect of cortisone on allogeneic inhibition in vitro, as detected by exposing sarcoma MACD of A × A.CA $F_1$ hybrid origin to antigenic extracts from strains A and C57BL, respectively. Findings are presented from three different experiments, where cells were cultivated in the absence or presence of cortisone.

| Cortisone ($\mu$g/ml) | Living cells ($\times 10^6$) after exposure to antigen from:* | | Inhibition by allogeneic antigen‡ (Av. No.) |
|---|---|---|---|
| | A† | C57BL† | |
| 0 | 2.10 | 1.32 | |
| 0 | 1.80 | 1.10 | |
| 0 | 1.78 | 0.91 | 1.57 |
| 0 | 1.74 | 1.29 | |
| 0 | 1.56 | 1.11 | |
| 2.5 | 1.92 | 1.49 | |
| 2.5 | 1.74 | 1.60 | |
| 2.5 | 1.54 | 1.60 | 1.05 |
| 2.5 | 1.50 | 1.53 | |
| 2.5 | 1.38 | 1.46 | |
| 0 | 1.88 | 1.12 | |
| 0 | 1.84 | 0.64 | 2.01 |
| 0 | 1.56 | 0.96 | |
| 0 | 1.48 | 0.64 | |
| 2.5 | 2.32 | 2.04 | |
| 2.5 | 2.00 | 1.67 | 1.16 |
| 2.5 | 1.68 | 1.36 | |
| 2.5 | 1.53 | 1.40 | |
| 0 | 2.56 | 1.80 | |
| 0 | 2.46 | 1.41 | 1.51 |
| 0 | 1.93 | 1.39 | |
| 2.5 | 2.46 | 1.73 | |
| 2.5 | 2.04 | 1.98 | 1.15 |
| 2.5 | 1.92 | 1.86 | |

*Antigenic preparations were made according to the technique of Haughton (*5*). Pooled antigenic material was diluted 1:2 in tissue culture medium, the final concentration being 0.3 mg antigenic material per milliliter culture medium. † Each entry represents the average number of cells unstained by trypan blue in a single culture tube after 48 hours growth in the presence of antigen. ‡ Number of cells in tubes given syngeneic antigen divided by number of cells in tubes given allogeneic antigen.

From the time of their appearance, tumors were measured by caliper, three diameters being estimated and the mean calculated.

Allogeneic inhibition could be demonstrated (Fig. 1) with both MC57S and MLG since there was a much higher tumor frequency in untreated syngeneic mice than in the $F_1$ hybrids. This is in agreement with earlier results (*2*). On the other hand, mice treated with cortisone before inoculation showed tumors in the same frequency and after the same latency period, irrespective of whether they were syngeneic with the tumors inoculated or whether they were $F_1$ hybrids.

Tissue-culture experiments were performed as described for allogeneic inhibition in vitro (*4*), except that cortisone was added to some of the cultures. The MACD tumor was used; this tumor is a fibrosarcoma originally induced by methylcholanthrene in an A/Sn × A.CA $F_1$ hybrid, and it has since been kept for 40 to 45 transfers in syngeneic A × A.CA $F_1$ hybrid mice prior to explantation. Stock cultures were prepared from MACD and used to start secondary cultures by inoculating roller tubes with $1.5 \times 10^6$ cells in 1.0 ml of Parker 199 solution containing 0.5 percent tryptose and 2 percent heat-inactivated horse serum. About 24 hours after they were seeded, the cells were exposed to antigenic extracts of A/Sn (syngeneic) and C57BL (allogeneic) origin, respectively. Antigenic extracts (*5*) were suspended in culture medium to two pools containing 9.4 mg of antigenic material per milliliter of medium. Dilutions (1:2) were made from these pools with tissue-culture medium, and 0.1 ml of diluted extract was inoculated into each tube. The same pools of antigenic extracts had been used for the demonstration of allogeneic inhibition in vitro (*4*). In these experiments antigenic extracts of A/Sn origin reduced the number of living cells in MC57S cultures (C57BL origin) but did not affect the MACD tumor, whereas the C57BL extracts had the reciprocal effect.

The possible effect of cortisone on allogeneic inhibition was studied by the addition of 2.5 $\mu$g of cortisone acetate (Upjohn) per milliliter of culture medium to half the number of tubes receiving a certain antigenic extract. The cortisone was given to the cultures at the same time as the antigens. The concentration used was not toxic in model experiments.

About 48 hours after addition of the antigenic extracts (72 hours after seeding of the secondary cultures), cell layers were detached from the glass surfaces by ethylenediaminetetraacetate. The number of living cells not stained by trypan blue was determined for each tube, and the ratios were calculated between cell number in tubes receiving syngeneic extracts and in tubes given "allogeneic" extracts.

Allogeneic extracts had a cytotoxic or growth-inhibitory effect as compared to syngeneic extracts, when no cortisone was added (Table 1). However, the degree of allogeneic inhibition decreased if cortisone was added together with the antigens. Thus, cultures given both antigen and cortisone had ratios of 1.05, 1.16, and 1.15, respectively, between cell number with syngeneic as compared to allogeneic extract, while these ratios were 1.57, 2.01, and 1.51, respectively, for tubes not given cortisone. The cell number in cultures receiving syngeneic extracts was not affected by cortisone.

It seems likely that abrogation of allogeneic inhibition by cortisone in vitro as well as in vivo has a common explanation. The findings in vivo may have been due to a depression of the immunological reactivity of the mice, but this is unlikely on the basis of the results in vitro and the absence of any influence of ordinary immunological reaction between host and graft upon allogeneic inhibition (*6*).

Abrogation of allogeneic inhibition by cortisone is reminiscent of the inter-

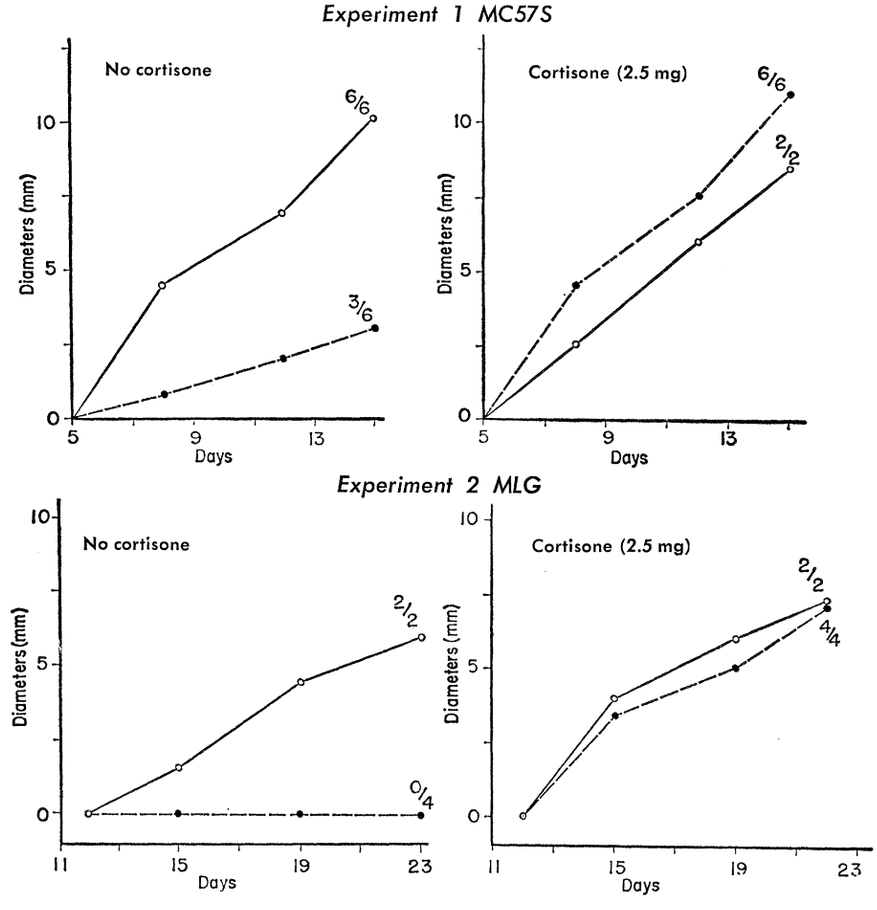


Fig. 1. Effect of cortisone upon allogeneic inhibition as demonstrated with sarcomas MC57S and MLG. Abscissae represent the number of days after inoculation and ordinates the mean tumor diameter in millimeters. Solid lines represent growth of tumors in syngeneic hosts and broken lines represent growth in semi-syngeneic $F_1$ hybrids. In experiment 1, MC57S was transplanted to syngeneic C57BL or semi-syngeneic C3H × C57BL $F_1$ hybrids. In experiment 2, the hosts for MLG were syngeneic C57L or semi-syngeneic A × C57L $F_1$ hybrids. Each curve is the average of a group of mice. The size of the group and the fraction of mice with growing tumors is shown for each group.

action of lymphocyte and target cells in tissue culture. Lymphocytes from already immunized allogeneic donors aggregate around appropriate target cells in vitro and kill them, whereas allogeneic lymphocytes from untreated animals neither aggregate nor kill (*7*). Unsensitized allogeneic lymphocytes are cytotoxic as well, provided that they are aggregated around the target cells, for example, by phytohemagglutinin (*8, 9*) or by a rabbit antiserum to mouse cells (*9*). When a cultured mixture of target cells and already sensitized lymphocytes was treated with cortisone, the lymphocytes still aggregated around the target cells but were no longer cytotoxic (*10*). Specific aggregation in the presence of cortisone indicates that antibody is still present at the surface of the lymphocytes, but the absence of killing suggests that the cytotoxic effect is not due entirely to fixation of such antibody. Possibly the cytotoxic effect of lymphocytes is due to the same mechanism (as yet obscure) that is responsible for the phenomenon termed allogeneic inhibition, and the function of specific antibody present at the surface of the lymphocyte may be to establish close contact with the target cells (*3, 9*). One would then postulate that cortisone either inhibits the ability of allogeneic antigens and target cells to interact or makes the target cells resistant to the cytotoxic effect of such an interaction.

K. E. HELLSTRÖM
I. HELLSTRÖM
G. HAUGHTON

*Department of Tumor Biology, Karolinska Institutet Medical School, Stockholm, Sweden*

### References and Notes

1. G. D. Snell and L. C. Stevens, *Immunology* **4**, 366 (1961); K. E. Hellström, *Nature* **199**, 614 (1963); K. E. Hellström, *ibid.* **201**, 893 (1964).
2. K. E. Hellström, *Science* **143**, 477 (1963).
3. ——— and G. Möller, *Progr. Allergy*, in press.
4. K. E. Hellström, I. Hellström, G. Haughton, *Nature* **204**, 661 (1964).
5. G. Haughton, *Transplantation* **2**, 251 (1964).
6. K. E. Hellström, in *Isoantigens and Cell Interactions, Wistar Inst. Symp. Monograph 3*, in press.
7. W. Rosenau and H. D. Moon, *J. Nat. Cancer Inst.* **27**, 471 (1961).
8. G. Holm, P. Perlmann, B. Werner, *Nature* **203**, 941 (1964).
9. E. Möller, *Science* **147**, 873 (1965).
10. W. Rosenau and H. D. Moon, *J. Immunol.* **89**, 422 (1962).
11. Supported by grants from PHS (C-3700 to G. Klein), Jane Coffin Childs Foundation, the Swedish Cancer Society, and the Swedish Medical Research Council. One of us (G.H.) is grateful to the International Union against Cancer for an Eleanor Roosevelt Fellowship. We thank G. Klein for support and Mrs. Solveig Humla, Barbro Janssen, and M. B. Sondell for technical assistance.

19 February 1965

## Nonspecific Binding of Complement by Digestion Fragments from Antiviral Gamma Globulin

Abstract. *The nonspecificity of rabbit γ-globulin (antibody) to western equine encephalitis virus and the nonspecificity of normal rabbit γ-globulin in complement-fixation tests with antigens prepared from chick-embryo cells infected with this virus and normal chick-embryo cells resided primarily in Porter's fragment III. Addition of complement to fragment III from the antibody globulin, followed by inactivation of the added complement, abolished the complement-fixing ability of fragment III with both specific and nonspecific antigens. Similar treatment of the undigested antibody abolished its complement-fixing ability with nonspecific antigen only.*

Taran (*1*) reported that the anticomplementary effects of an antiserum could be abolished by the addition of complement (C′) to the serum at 37°C and by then heating the mixture to 56°C to inactivate the added C′. When added to fresh C′ such treated antiserum no longer showed anticomplementary activity, but when mixed with its specific antigen it bound C′ as would be expected.

We report the results obtained with fragments of normal rabbit γ-globulin and that from rabbits immunized with western equine encephalitis (WEE) virus in the aforesaid system. The preparation of the antiserum pool, isolation of γ-globulin, and the preparation and isolation of papain-digestion fragments have been described (*2*); however, fragment III was further purified by recrystallization two to three times in the cold. The pepsin-digestion fragment was obtained by the method of Nisonoff *et al.* (*3*). The papain digest (in 0.1*M* acetate buffer, *p*H 5.5) on analytical centrifugation showed one peak at 3.3*S* and the pepsin digest (in borate-buffered saline at *p*H 8.0, ionic strength 0.16) showed one main peak at 4.3*S* plus a small amount of slower-moving material (56,100 rev/min, 20°C, 0.7 percent protein). There was no evidence of a faster-moving component in either preparation. All products showed a single line by Ouchterlony analysis when checked with goat antiserum to rabbit γ-globulin; papain-digestion fragments I and II and the pepsin digest all showed identity with each other, whereas fragment III differed from all the others. All products showed a cross-reaction with the undigested γ-globulin. The complement-fixing antigen in the encephalitis virus was prepared from infected chick-embryo tissue culture by a method described for herpes simplex virus (*4*), with the exception that just prior to the C′-fixation test the cell debris from the crude tissue-culture lysate was removed by centrifugation. Thus, the virus antigen was essentially a crude extract of infected chick-embryo cells plus the culture medium (2 percent lamb serum, 0.5 percent lactalbumin hydrolyzate, 97.5 percent Eagle's basal medium prepard in Hanks balanced salt solution). The antigen used for specificity control was prepared from normal chick-embryo tissue culture in the same manner as for the virus antigen. The complement-fixation tests were performed by a modification (*5*) of the Takatsy (*6*) microtitration, with Kolmer's saline as diluent. The results were recorded as 0, 1+, 2+, 3+, and 4+, according to the degree of inhibition of hemolysis in the indicator system. End points, based on presence (size) or absence of a "button" of red blood cells plus the degree of hemolysis in the supernatant fluid, were read with the naked eye. Results in Tables 1 and 2 are expressed as reciprocals of the highest initial dilution giving at least a 2+ reaction, corresponding roughly to 50-percent hemolysis as end point. All protein samples were heat-inactivated at 60°C for 30 minutes prior to testing.

Samples (0.15 ml) of those preparations showing complement fixation were mixed with the rehydrated solution of lyophilized guinea pig serum (0.05 ml) and incubated at 37°C for 30 minutes. This mixture, after a fourfold (0.6-ml) dilution with Kolmer's saline, was incubated at 60°C for 30 minutes to inactivate the guinea pig complement. The complement-fixation test was then repeated with these treated samples, with active guinea pig serum and virus antigen or normal control antigen (Tables 1 and 2). The same results were obtained when the procedure was reversed—for example, when complement was heated to 60°C for 30 minutes, then added to the sample, and further incubated at 37°C for 30 minutes.

The fragments I and II of antibody γ-globulin to the virus, which contain one antibody-combining site (*7*), and the pepsin-digestion fragment, which