References and Notes

1. The following abbreviations are used: ADP, adenosine diphosphate; P_i, orthophosphate; DNP, 2,4-dinitrophenol; and ATP, adenosine triphosphate.
2. L. Schachinger, R. H. Eisenhardt, B. Chance, *Biochem. Z.* **333**, 182 (1960).
3. R. H. Eisenhardt and O. Rosenthal, *Federation Proc.* **21**, 56 (1962); R. H. Eisenhardt, abstr., 145th meeting, Am. Chem. Soc., New York, Sept. 1963, p. 63-C.
4. R. H. Eisenhardt and J. C. Schuder, abstr. 137th meeting, Am. Chem. Soc., Cleveland, Ohio, April 1960, p. 5-C.
5. P. V. Vignais, abstr. 145th meeting, Am. Chem. Soc., New York, Sept. 1963, p. 63-C.
6. R. Butow, personal communication.
7. Limitation of the reaction rate by slow diffusion of P_i through mitochondrial membranes is eliminated in submitochondrial particles.
8. E. Racker, *Advan. Enzymol.* **23**, 323 (1961).
9. B. Hagihara and H. A. Lardy, *J. Biol. Chem.* **235**, 889 (1960).
10. Supported by grant CA-6266 from the National Cancer Institute. The assistance of Tatiana Schebalin is acknowledged.

14 November 1963

Growth Inhibition of Sarcoma and Carcinoma Cells of Homozygous Origin

Abstract. *Three sarcomas and one carcinoma, originating in homozygous mice, were tested for growth in isologous animals and various semi-isologous F_1 hybrids after inoculation of 10^3 to 10^5 cells. Findings indicated the existence of an "F_1 hybrid effect" for sarcomas and carcinomas, as previously described for lymphomas and normal hematopoietic cells, there being a lower tumor frequency and a longer latency period in the hybrids than in isologous mice.*

In an earlier report I described the differential behavior of transplanted lymphoma lines in genetically compatible homozygous and F_1 hybrid mice (*1*). Isoantigenic variant sublines were studied, isolated from lymphoma LNSF of A × A.SW F_1 hybrid origin by selective passage in one of the parental strains, A or A.SW. Subsequent to such passage, they became specifically compatible with the parental strain of selection and had lost, apparently irreversibly, the H-2 isoantigens specifically derived from the opposite parental strain. When compared with the original LNSF line in the F_1 hybrid type of origin (A × A.SW), it was found that the variants grew more slowly. In the parental strain where they have been selected they grew faster than in the hybrid and behaved in the same way as the unselected line in the F_1 hybrids. Although the mechanism of this phenomenon is not clear at present, it can be attributed to the H-2 antigenic loss characterizing the variant cells and the resulting difference between graft and host when they are inoculated into F_1 hybrids. It is probably analogous to the finding of Snell that homozygous lymphomas grow better in mice of their strain of origin than in various F_1 hybrids (*2, 3*). The possible existence of a similar F_1 hybrid effect with normal hematopoietic cells is indicated from studies by Popp (*4*), McCulloch and Till (*5*), and Cudkowicz and Stimpfling (*6*) on homozygous cells transplanted to lethally irradiated mice of various F_1 hybrid genotypes and to irradiated homozygous mice of the isologous strain. On the other hand, no differences have been found in the survival of skin grafts from homozygous mice transplanted to the same strain and to various genetically compatible F_1 hybrids (*7*). It appeared, therefore, essential to know whether the F_1 effect is restricted to normal cells of the antibody-forming system and their malignant counterparts, or whether it is a general phenomenon; the first alternative would substantiate the suggestion of Snell and Stevens that the F_1 effect found by them with homozygous lymphomas is due to "an abortive graft versus host reaction" (*3*).

In the present work an attempt has been made to demonstrate an F_1 hybrid effect with three methylcholanthrene-induced fibrosarcomas, two of which were of C57 BL/Kl (MC57 G and MC57 S) and one of C57 L/Kl origin (MLG), and with one spontaneous mammary carcinoma of A.CA origin (S2C). The tumors were serially propagated by subcutaneous transfer in mice of their strain of origin. Trypsinized cell suspensions were prepared in a way similar to that for tissue culturing and inoculated subcutaneously in a volume of 0.1 ml containing 10^3, 10^4, or 10^5 eosin-unstained cells into homozygous mice of the strain of tumor origin and to various genetically compatible F_1 hybrids, as shown in Fig. 1. All mice inoculated were of the same weight (16 to 18 g), and approximately the same proportion of males and females were used in the experiments. The mice were inspected every 2nd to 4th day after inoculation, and the time required for the appearance of a tumor was recorded, as well as the growth rates of the tumors.

The pooled results (Fig. 1, *A–D*) demonstrate that all tumors grew better in mice of their strain of origin than in different semi-isologous F_1 hybrids. As already found for lymphoma cells (*1*), different F_1 hybrids varied in their capacity to support tumor growth. The C57 BL sarcomas grew regularly better in A.CA × C57 BL F_1 mice than in A × C57 BL, DBA × C57 BL or C3H × C57 BL F_1 hybrids. The difference between homozygous and F_1 hybrid mice was apparent both with regard to latency period preceding tumor appearance and total tumor frequency, whereas no certain differences were detected in the growth rates of established tumors. As with lymphoma cells, the "F_1 effect" did not increase after prior immunization of the mice (*2, 3, 8*) and the effect remained after irradiation of the mice with 450 roentgens prior to grafting (*1*).

Thus the previously described F_1 effect is not restricted to cells of the antibody-forming system and their malignant counterparts. As a corollary, it is not likely to be caused by an abortive

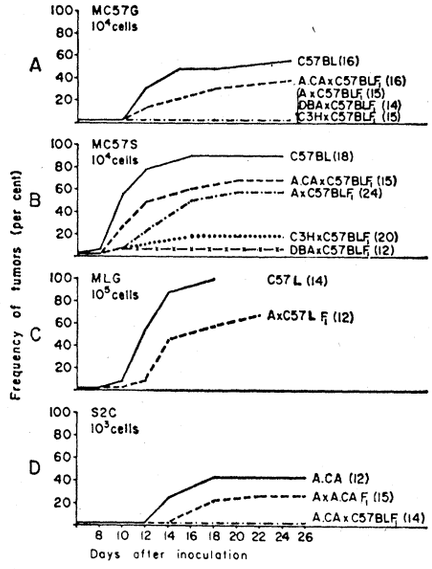

Fig. 1. Inoculation of four different tumor lines, derived from and carried in homozygous mice, to homozygous mice of the strain of tumor origin and various semi-isologous F_1 hybrids. The number of mice inoculated in each group is shown within parentheses. The data are pooled from three separate experiments with each tumor line. *A*, Tests with MC57 G, a methylcholanthrene-induced fibrosarcoma in C57 BL, serially carried during 16 to 18 passages before the tests (10^4 cells were inoculated). *B*, Tests with MC57 G, a methylcholanthrene-induced fibrosarcoma in C57 BL, serially carried during 12 to 16 passages before the tests (10^4 cells inoculated). *C*, Tests with MLG, a methylcholanthrene-induced fibrosarcoma in C57 L, serially carried during 28 to 30 passages before the tests (10^5 cells inoculated). *D*, Tests with S2C, a spontaneous mammary carcinoma in A.CA, serially carried during 39 to 41 passages before the tests (10^3 cells inoculated).

graft versus host reaction. It remains to be shown conclusively for sarcomas and carcinomas, however, as has already been done for lymphomas, that the F_1 effect is really due to the difference at the H-2 locus between tumor and host. This can be achieved, for example, by using isoantigenic variant sublines isolated from tumors induced in F_1 hybrids between two isogenic resistant strains (*1*).

The absence of an F_1 effect detectable by skin transplantation may seem surprising in view of the present findings and this perhaps depends on differences in cell numbers inoculated (large in the case of a skin graft, small in the relevant experiments with tumor cells). Other differences are that hematopoietic and tumor cells are inoculated as suspensions of free cells, less protected than cells within a skin graft, and the fact that the tumor and hematopoietic cell populations studied grow logarithmically, while cell number is more stationary within a skin graft.

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References and Notes

1. K. E. Hellström, *Nature* **199**, 614 (1963).
2. G. D. Snell, *J. Natl. Cancer Inst.* **21**, 843 (1958).
3. ——— and L. C. Stevens, *Immunology* **4**, 366 (1961).
4. R. A. Popp, *J. Natl. Cancer Inst.* **26**, 629 (1961).
5. E. A. McCulloch and J. E. Till, *J. Cellular Comp. Physiol.* **61**, 301 (1963).
6. G. Cudkowicz and J. H. Stimpfling, *Genetics* **48**, 886 (1963).
7. O. Linder and E. Klein, *J. Natl. Cancer Inst.* **24**, 707 (1960).
8. Unpublished results.
9. Supported by grants from the Swedish Cancer Society and the Swedish Medical Research Council and by grant C-3700 to Prof. G. Klein from the National Cancer Institute, U.S. Public Health Service. Thanks are due Prof. Klein for support and to Miss Barbro Jansson for technical assistance.

12 November 1963

Hormonal Activation of the Insect Brain

Abstract. *A new endocrine function has been shown to play a role in insect morphogenesis. The hormone named* proctodone *is secreted by specialized cells of the hindgut and appears to affect the brain, resulting in the activation of the brain hormone-producing system. Proctodone has been found in two lepidopterous species*—Ostrinia nubilalis *and* Galleria mellonella. *In the* O. nubilalis, *proctodone plays a part in both photoperiodism and diapause.*

Neurosecretory centers in the insect brain are thought to initiate the complex physiological processes leading to growth and molting. Diapause, as a state of arrested growth, is considered to be caused by an endocrine failure, usually that of neurosecretion; and the resumption of neurosecretory activity signals the termination of the diapause state (*1*). There is increasing evidence, however, that diapause is not invariably produced by a direct effect of genetic and environmental factors on the neurosecretory cells, but may be the result of changes in unidentified physiological processes to which neurosecretory processes are coupled (*2*).

Most of our experiments were with larvae of the European corn borer, *Ostrinia nubilalis*, in which larval diapause had been induced by short-day photoperiods (12 hr light/day). Borer larvae have been shown to be sensitive to photoperiod while in the diapause state, and diapause may be terminated by exposing the larvae to long-day photoperiods (16 hr light/day) for 10 to 12 days (*3*). According to the currently accepted concepts of insect growth, the larval brain is, in some unidentified way, reactivated, and the larvae resume morphogenesis leading to pupation.

When borer larvae in diapause were effectively bisected by the application of ligatures between the 6th and 7th abdominal segments, exposure to long-day photoperiods did not lead to the termination of diapause. Ligation at the 9th abdominal segment did not prevent diapause termination. From such experiments, it was concluded that the termination of diapause depended upon some event occurring in the 7th or 8th abdominal segments. All of the known endocrine organs are found in the head and thorax, and the photoperiodic response is also thought to originate in the cephalic parts of the insect (*4*). According to the known endocrine functions, abdominal ligations should not have prevented pupation of those portions of the larvae lying anterior to the ligatures.

A series of experiments, in which diapause larvae were exposed to long-day photoperiods for different intervals before the application of abdominal ligatures, demonstrated that the action of the abdominal system was required for a period of 10 to 12 days after the beginning of the long-day treatment. Ligations after this time did not prevent the termination of diapause, and the anterior portions of the ligated larvae pupated. Further ligation experiments demonstrated that the endocrine activity of the brain was required for several days after the abdominal system had completed its required function.

The experimental results strongly suggested that the function of the abdominal system was to activate the endocrine organs associated with the brain. This might be accomplished in either of two ways: (i) by nerve impulses arising in the posterior ventral ganglion in response to long-day photoperiods; or (ii) by hormones being produced in the 7th or 8th abdominal segments, with such production being regulated by photoperiod. The first of these alternatives was eliminated by the results of surgical experiments. Severing the ventral nerve cord at the 6th abdominal segment did not prevent the photoperiodic termination of diapause.

Table 1. Effect of tissue implants and abdominal ligations on morphogenesis of diapausing larvae of the European corn borer.

Material implanted or injected	Condition of larvae	Photoperiod	Larvae treated (No.)	Mortality (No.)	Pupae* (No.)
A. Diapause brain	Diapause and brainless	Long-day	12	1	11
B. Diapause brain	Diapause and abdominal ligation	Long-day	20	8	0
C. Nondiapause brain	Diapause	Short-day	18	0	18
D. Nondiapause brain	Diapause and abdominal ligation	Short-day	18	4	12
E. Proctodeal extract	Late diapause	Short-day	29	2	17
F. Saline control	Late diapause	Short-day	18	7	3

* Number pupating within 20 days after treatment.