Immunological Enhancement of Tumor Homografts in Mice

A Review*

NATHAN KALISS

(Rosecr B. Jackson Memorial Laboratory, Bar Harbor, Maine)

The term "immunological enhancement" (28, 31) applies specifically to the successful establishment of a tumor homograft and its progressive growth (usually to death of the host) as a consequence of the tumor's contact with specific antisera in the host. The presence of the antisera is effected either by active immunization with tissues from the strain of mice to which the test tumor is indigenous or by passive immunization with hetero- or isoantisera (25, 26, 34, 35, 43). Conceivably, the term could be applied to the experimentally prolonged survival of homografts of normal tissues and heterografts of either cancers or normal tissues, if the operative conditions meet the requirements as defined above.

(It is not the intent of this article to present a review of the earlier literature on enhancement and apparently related phenomena. This literature has been covered in [24, 56].)

The apparent paradox of immunological enhancement is that the procedures for obtaining it are the same as those that would ordinarily be used for inducing heightened resistance to a homograft (as was first pointed out by Snell [50]). Thus, the best results are obtained if the prospective host is treated with the same tumor tissue as the test homograft (57) or with normal tissues or other transplantable tumors from mice of the inbred strain indigenous to the test tumor (36, 49). The tissues may be injected as saline homogenates of fresh, frozen, or freeze-dried tissues, or of supernatants of such suspensions (11, 30, 37, 49). With homogenates, the state of the tissues appears to be of little consequence, at least for the one test system—the strain A tumor Sarcoma I in C57BL/Ks hosts—which we have used the most. However, repeated freezing and thawing appear to increase the effectiveness of supernatants (Kandutsch, unpublished data). The injections must precede grafting, but the effect of the treatment (enhancement) is demonstrable with tumors inoculated as long as 46 weeks after the administration of the tissues (Chart 1) (32).

A dosage phenomenon is demonstrable for some graft-host combinations (in which the grafts will grow in about 50 per cent of untreated controls), very small doses of freeze-dried tissue inducing heightened resistance in the host, while large doses permit enhancement (23). It is now well established that it is antigenic identity (determined by genetic identity) between the test tumors and the preparatory tissues which underlies these observations (51), a point which will be elaborated below.

Enhancement can be induced with passively transferred antiserum which has been produced in rabbits or in mice immunized with normal...
or cancerous mouse tissues (25–27, 34, 35). The effectiveness of the serum is due to the presence of specific antibody, and not to the continued presence of some substance introduced into the rabbit or mouse with the immunizing tissues (25-27, 34). The activity of the antiserum is associated with the globulin fractions, and most probably with γ-globulin (34). In contrast to the long persistence of the enhancement response in mice injected with tissues (see Chart 1), there is a rapid dropping off in effectiveness of passively transferred antiserum as the time interval between injection of serum and the subsequent inoculation of the graft is increased (Chart 2) (25).

Antiserum injected AFTER tumor grafting.

Antiserum injected BEFORE tumor grafting.

CHART 2.—Passively transferred heteroantiserum (produced in rabbits) or isoantiserum (produced in C57BL/Ks mice) will enhance inocula of Sarcoma I in otherwise untreated C57BL/Ks hosts. The best effect is obtained if the antiserum is injected shortly before or shortly after tumor inoculation.

The modus operandi of immunological enhancement is the exposure of the tumor graft to specific humoral antibody.—This postulate is consistent with the observations that have been listed above. It is further supported by experiments in which cortisone was administered to the prospective host during the period of active immunization. It has been found that hemagglutinin production (33) and enhancement are suppressed under these conditions, but if antiserum is supplied by passive transfer to mice similarly treated, successful enhancement follows (Chart 3) (27). This postulate explains why the enhancing effectiveness of passively transferred antiserum falls off with time (as the concentration of circulating antibody declines) and why the rate of “decay” is more rapid with heteroantiseras (Chart 2). It explains why enhancement is possible many months after a mouse has received a preparatory injection of tissue or a live tumor inoculum (Chart 1). Effective enhancing antiserum can be obtained from such mice throughout this period (26), and these sera also exhibit a good hemagglutinating titer (Chart 4) (sera kindly titered for us by Dr. D. B. Amos).

It is probable that “enhancing” antibody and hemagglutinating (H-2) antibody are engendered by the same antigens and are the same moiety of the serum, but the evidence for this, though suggestive, is not conclusive. Hemagglutinating activity (1) and enhancing activity (34) are both associated with the gamma-globulin fractions. There is a positive correlation between the hemagglutinin titer and enhancing potency of an anti-tumor serum. Both of these properties have been appreciably lessened by successive absorptions of the serum with red blood cells (from the inbred strain indigenous to the test tumor) and completely removed after absorption with the tumor tissue (unpublished data). Snell (51) has adverted to the correlation between the known H-2 antigenic composition of a given host-tumor graft combination and the likelihood that enhancement would, or would not, take place. On the basis of the known H-2 antigenic constitutions of different inbred strains of mice, it is possible to predict whether or not enhancement of the strain A tumor Sarcoma...
I will follow the injection of a given isoantiserum. We have found (unpublished data) that at least three different H-2 antigens, D, E, and K, may be involved in the enhancement reactions of Sarcoma I, and it is possible that this list would be extended if the necessary types of inbred strains of mice were available. (For example, in collaborative experiments with Dr. P. A. Gorer, to be discussed in further detail below, enhancement of the C3H tumor B.P. 8 [H-2K] in strain A mice [H-2A] was obtained with passively transferred A anti-B.P. 8 serum. Since, as far as is known presently, the H-2A antigenic complex contains all the antigens present in the H-2K complex, it is not clear which antigens, peculiar to the C3H strain of mice, are involved in this enhancement reaction. These might be allelic forms of antigens D or F [2, 14, 21], or M and N [Amos, unpublished data], or other as yet undetermined H-2 antigens [or other than H-2 antigens].) The importance of D and K has been pointed out by Snell (51). It is recalled that the H-2 antigens, which are present in many (if not all) tissues of the mouse, have been determined serologically, chiefly by hemagglutination (2, 21). Though these findings all point to an identity between the classical H-2 antibodies and "enhancing" antibody, the possibility remains, until proved otherwise, that they are different entities, generated in response to the same antigens. Final clarification of this point will have to await the successful isolation and characterization of the responsible antigens, which are being carried out by Kandutsch and his co-workers (37).

(It is possible that antigens other than the H-2 type, which Kandutsch believes may be mucoproteins [37], can initiate immunological enhancement. There are reports of a "mammary tumor accelerant" which appears to be a lipide [41] and of a nucleoprotein inducing enhancement of the Ehrlich ascites tumor [22]. It is not clear whether immunological enhancement is involved in these instances, since there were no attempts to test for the possible presence of enhancing antisera. It is also open to serious question whether the activity of the so-called "nucleoprotein" preparation is not owing to contaminants other than nucleoproteins.)

**Chart 3.**—Cortisone injected into the prospective host (C57BL/Ks mice) during the period of active immunization with Sarcoma I supernatant, suppresses enhancement of Sarcoma I grafts. Enhancement is produced if antiserum is also injected, at the time of tumor grafting, into the mice treated as above. (Vertical axis shows the materials injected in each group of mice; horizontal axis, the proportion of mice dying with enhanced tumors. Figures in parentheses at the end of each bar are: number of mice dying with tumors/total number of mice in the group.)

**Chart 4.**—The hemagglutinin titer (vertical axis) of C57BL/Ks anti-Sarcoma I sera, taken at different time intervals (horizontal axis) after the inoculation of a live graft or a single injection of freeze-dried tumor (sera titered by Dr. D. B. Amos).
Enhancement of tumor homografts by procedures that appear to be comparable to those used for immunological enhancement have been reported by other investigators for the rat (19), rabbit (9), and mouse (9, 22). Apparently, similar observations have been made with "isografts" of mouse tumors (41, 48) (if immunological enhancement is the mechanism responsible for the results in this instance, then it must be assumed that it was due to some degree of antigenic incompatibility between the host and its nominally indigenous tumor graft). However, of all these reports, only that of Green and Wilson (19) states that enhancement was produced (in the rat) with passively transferred antiserum. This procedure was not tried by the other investigators. Pikovski and Schlesinger's demonstration (46) of the enhanced growth of mouse tumors in the rat pre-treated with mouse tissues possibly may be more akin to immunological tolerance (in Medawar's sense [40]) than to immunological enhancement. Thus, these investigators found that continued progressive growth of the heterografts was obtained only in rats receiving the preparatory tissue injections at an early age. These authors did not carry out any serological determinations and did not try the effects of passively transferred antiserum, studies which are critical for establishing whether or not they were dealing with immunological enhancement.

Whether accelerated destruction of a homograft or, conversely, its enhancement, is the consequence of immunizing the prospective host depends upon a number of variables, some of which have been defined, while others are still not understood. The interaction of these variables determines the "host-graft relationship" in a given combination of tumor and host, and the expression of this relationship appears to depend upon inherent differences in the way both the host and the graft respond to the experimental procedures. The material that follows illustrates the operation of some of the variables affecting enhancement in inbred mice, where they can be studied most easily.

A subcutaneous graft of the strain A tumor Sarcoma I in C57BL/Ks mice sets off a train of immunologic reactions which is initially characterized by a heightened resistance of the host to a subsequent inoculum of the tumor (31). The intensity of the rejection reaction subsides with time, and eventually it is possible to produce enhancement of a second graft. The temporal relationships of these converse reactions have been delineated by using the fate of a second tumor graft as an index of the nature of the host's immune responses as induced by a first graft of the same tumor line. In summary, these experiments show that the first evidence of heightened resistance is present by 3 days after the first grafting, reaches maximum intensity by 5 days, and begins to subside by 2-8 weeks. Enhancement now becomes possible in a limited number of the second grafts, and it is almost always produced if the interval between graftings is longer than 1 month. The appearance of enhancement coincides with the waning of the rejection reaction and the concomitant increase and sustained production of humoral ("enhancing") antibody (Chart 5) (26, 31). (Our data are in accord with those of Mitchison [42], who found the same time relationships for the waxing and waning of graft resistance and the production of hemagglutinating antibody as mediated by passively transferred lymphoid tissue taken from immunized mice.)

A similar duality of response has been demonstrated by the same experimental design with the strain A tumor 15091a in C57BL/Ks hosts. Of interest was the much longer lasting resistance of the females, enhancement of the second graft only appearing when the interval was at least 16 weeks between successive inoculations, while in the males the necessary minimum period was 4-8 weeks. The higher level of the immune response in the females is also shown very strikingly in the much higher initial titers of hemagglutinating antibody produced in response to a single inoculum of 15091a (28), though after several months the titers decreased to about an equal level in both sexes (unpublished data—titers kindly determined for us by Mrs. Z. B. Mikulska).

Whether or not enhancement will take place depends in part upon the particular host-tumor combination used (57; Kaliss, unpublished data). Thus, of the two strain A tumors, Sarcoma I and 15091a, enhancement is more readily and consistently produced with the former. With respect to a third strain A tumor, C1300, enhancement is readily produced with passively transferred isoantiserum, but only very rarely following active immunization (unpublished data). In fact, until recently, enhancement after active immunization was never possible with this tumor, and the current slight success may demonstrate some change in the tumor. With respect to the host, enhancement of Sarcoma I in actively immunized animals comes most readily in C57BL/Ks (H-2D) or C57BL/10-H-2d mice, and less readily in mice of the C57BL/6Jax (H-2B) or C3H/Ks (H-2K) strains. It is possible that these differences depend in part on the particular H-2 antigenic category of the strains, but this cannot be the sole explanation. Thus, within the H-2K group, enhancement is much more readily producible in the C57BR/cd strain, as compared with the C3H/Ks strain. In
physiological terms, the strain differences may be the expression of either a more intense and longer enduring high level of resistance, or of a lower level of production of “enhancing” antibody. That a sustained high level of resistance is the more probable explanation is shown by the occasional C57BL/Ks mouse (particularly among the females) that remains resistant to repeated inoculations of Sarcoma I (in contrast to the more usual experience of enhancement of a second graft inoculated at a sufficiently long interval after the first). Such a mouse, nevertheless, is producing enhancing antibody whose effectiveness can be demonstrated on passive transfer to another C57BL/Ks mouse (31).

The maintenance of an irrevocable rejection reaction, with a well marked “second-set response,” is illustrated in C3H/Ks mice (H-2K) receiving as many as five successive inocula of Sarcoma I, with intervals of 11 weeks to 3 months between each two inoculations (31). The first graft often will grow to a very large size before regression (much larger than in untreated C57BL/Ks mice). These “refractory” animals, however, are producing enhancing antibody, as can be shown on passive transfer of their sera to untreated C3H/Ks mice (or to mice of other H-2K inbred strains [unpublished data]). Furthermore, enhancement of Sarcoma I is possible if the initial immune stimulus is in the form of a tumor extract rather than an inoculum of live tumor. It may be assumed that the immune response to the extract is expressed predominantly in the production of humoral (enhancing) antibody rather than the cellular response associated with graft resistance (as has been shown by Mitchison [42]), so that enhancement is now realizable.

The manner in which immunological enhancement is expressed may depend upon the site of inoculation of the graft and the dosage of passively transferred serum. This is brought out in a very interesting fashion in experiments we have conducted with Dr. P. A. Gorer in his laboratory at Guy’s Hospital.1 The test tumor

1P. A. Gorer and N. Kaliss, The Effect of Isoantibodies in Vivo on Three Different Neoplasms in Mice (to be published).
was the C3H sarcoma, B.P.8 (H-2K) (maintained in the ascites form), and the hosts were of the BALB/c strain (H-2D). The mice received different dosage levels of BALB/c-anti-B.P.8 serum at the time of tumor inoculation. A uniform dosage of tumor cells was injected into the right calf muscle, this locus being chosen because it was found to give more consistent results than the subcutaneous site. The data for one such experiment are graphed in Chart 6. The curves show the change in average size of the growths with time in each group of mice, the groups being differentiated by the amount of serum injected per mouse. The striking feature of these experiments is that the best enhancement, with respect to both the rate of growth of the grafts and the number of animals dying with tumors, was obtained in the groups of mice receiving the lower dosage of serum. In another experiment with B.P.8, in which both the hosts and the source of the isoantiserum were strain A mice (H-2A), the results again were enhancement at the lower serum dosage (0.05 ml.) (actually a delayed regression, for all the grafts eventually regressed). However, at the highest dosage of serum (0.2 ml.) there was a definite inhibition of the grafts, as compared with the untreated controls. With the same tumor and a third strain of mice, C57BL/Go (H-2B), acting both as host and source of the enhancing serum, the results were again different. There was a marked enhancement with both the large (0.2 ml.) and small (0.05 ml.) doses of serum, and no difference was found between the two groups in rate of growth of the grafts. All the enhanced tumors eventually regressed, however, though they grew to a size which in BALB/c mice was invariably associated with continued growth of the graft and consequent death of the host.

The biology of immunological enhancement is clearly different from that of "acquired tolerance" or "immunological paralysis," though each of the three terms describes what superficially appears to be the experimental abrogation of an animal’s normal immunologic reactivity as a result of the injection of antigen. The operational differences have been summed up clearly by Medawar (40), and the reader's consideration is referred to his discussion. We shall concern ourselves in detail only with graft enhancement, which requires humoral anti-graft antibody for its realization. How does contact between graft and antiserum effect enhancement? A categorical answer is not possible as yet, and it is certainly much more difficult to state what the consequences of the contact are that eventuate in enhancement, than what they are not. Several possibilities have been ad-
of two factors: (a) such mice produce only humoral hemagglutinins (since the "transplantation antigens" are rendered inactive in the process of preparing the tissues [6]); (b) the antigenically competent "transplantation antigens" introduced with the subsequent inoculation of the test graft cross-react with the hemagglutinins and thereby are "inactivated"; i.e., they are now incapable of providing the immune stimulus which otherwise would have induced graft resistance. Step b would apply alone in the case of passively transferred "enhancing" (hemagglutinating?) antibody. This ingenious formulation may appear at first sight to stand or fall on the validity of the presumed demonstration of two categories of antigens. It is not the function of the present article to examine this question (however, see [54]), but even if it were shown unequivocally that there are two types of antigens, any hypothesis of blockage still is untenable in the face of our own data, which will be considered in further detail below. (It has been shown in other connections [58] that an antigen-antibody complex can provide a competent immune stimulus, though the manner in which the host responds is somewhat altered.) Snell's formulation (52, 54) differs from Medawar's in two respects. It does not invoke the presence of two different classes of antigens, and apparently it does not invoke "inactivation" of the antigens. What seems to be adduced is a literal "walling off" (fixation) of the homografted tumor cells by humoral antibody, so that the cells, with their antigens, are prevented from getting to the host's immunologically reactive centers (e.g., the draining lymph nodes) and thus cannot induce a rejection response. Snell cites supportive evidence for this hypothesis from experiments in which minced normal lymph nodes, obtained from inbred mice of the strain to which the test tumor is indigenous, were mixed with the tumor cells just prior to inoculation into a pretreated mouse. This procedure led to inhibition of the graft, while enhancement was observed in the mice receiving tumor cells without an admixture of lymphoid tissue. Presumably, the highly mobile lymphocytes could not be "fixed" by antibody and thus could get where they would impart the necessary immune stimulus for the transplantation reaction. Now this observation, intriguing in itself, does not necessarily mean that the chain of events set off in the host has anything to do with enhancement per se. An analogous example would be our finding that cortisone inhibits enhancement (27), not possibly owing to any inhibition of antigen-antibody reactions, but because it suppresses the production of the necessary humoral antibody. There are other difficulties with the "walling off" hypothesis. The result with the lymphocytes (52, 54) does not of necessity imply that there is any "walling off" of antigens in the tumor graft, but, even if it is granted that there is a walling off, it cannot be concluded perforce that this serves to protect the graft. On the contrary, if a parallel were drawn with the events in microbial infections (7, 8), then "walling off" should serve to insure and accelerate destruction of the "opsonized" graft.

It is necessary, at this point, to reiterate and elaborate on some of the data which bear on the validity of any hypotheses of "blockage." Our experiments with successive inoculations of live tumor (29, 31) show (as did Mitchison [42]) that a first graft evokes a state of heightened, immunologically specific resistance to a subsequent graft, the resistance reaching peak intensity by 5 days after inoculation of the tumor, and beginning to subside by about 14 days. (These figures are based on our experience with Sarcoma I in C57BL/Ks mice.) Nevertheless, in a proportion of animals receiving a single tumor inoculum, it is possible to induce enhancement by injecting antiserum into an otherwise untreated mouse as long as 7-10 days after grafting (Chart 2) (25). In other words, enhancement is possible in an animal which has been subjected to the immune stimulus of the "transplantation antigens" and has had the opportunity to develop a full rejection response. In animals receiving two successive tumor inoculations, it is not unusual to find that the first inoculum may show a marked initial regression and then a "revival," with continued growth towards death of the host (Chart 5) (31). The second graft, meanwhile, has been rapidly destroyed by the "second-set response," even though enhancing antiserum may have been injected at the time of the second inoculation. It may be assumed that the heightened antibody level, due either to passively transferred antiserum or to a secondary immune response to the second graft, has helped the first graft to cross the "enhancement threshold" and achieve progressive growth, but it could not save the second graft from the consequences of the heightened (second-set) rejection response.

The following histological observations are pertinent to this problem. P.R.F. Borges (cited in [54]) describes preliminary studies of the histological sequence of events in the subcutaneous bed of Sarcoma I homografts in C57BL/Ks or C57BR/cd hosts. Some of the hosts had been actively immunized with injections of tumor supernatant (in the usual enhancement procedure). In the early stages after inoculation of the graft,
the cellular reactions in the pretreated mice and untreated controls were indistinguishable. Recently, Gorer (15, 16) has made detailed studies of the reactions of BALB/c mice receiving subcutaneous grafts of Sarcoma I and a simultaneous injection of BALB/c anti-Sarcoma I antiserum. In agreement with Borges' findings for the actively immunized mice, there was "... no sign of delay in the time of onset of the homograft response" (which developed as a histiocytic response) in the serum-treated mice (16). At the 7th day after inoculation of the tumor, the difference between the experimental and control groups becomes evident as a violent interaction between histiocytes and tumor cells in the controls, while in the experimental mice the cellular response fades away and there is "... a violent increase in mitotic activity in the tumor." The histological data thus give no comfort to a hypothesis of antigenic blockage. It may be argued that the initial histiocytic response is a nonspecific, foreign-body response. However, such responses are not seen with indigenous (isologous) tumor grafts (Gorer, personal communication), and the blockage hypothesis implies that the "inactivated" antigens do not provide an immune stimulus and therefore should not be expected to provoke any "foreign" response in the host.

We have experiments in progress in which two successive inoculations of Sarcoma I ascites cells have been made in C57BL/Ks mice pretreated with tumor supernatant, in the usual enhancing procedure. The tumor inoculations were spaced 1 week apart, the first one being administered 8 weeks after the start of the supernatant injections. The point of interest is that there was an unequivocal second-set response in all of these mice, while enhancement of the first inoculum took place in a significant number of animals. It is thus clear that the homograft reaction is not blocked by the enhancement procedure, a conclusion which is diametrically opposite to Mitchison and Dube's (43). It is emphasized that there is not necessarily a contradiction in the data, for the experimental designs are quite different in the two instances. Mitchison used the method of transfer of lymph node material to study the nature of the immune response, as revealed in the recipients of the nodes, while we were studying the response directly in the treated animal.

That these different approaches may reveal different kinds of responses is shown by other experiments of the type just quoted above, but in which we also administered cortisone throughout the period of injection of tumor supernatant, and up to the time of the second inoculation of tumor cells (unpublished data). These mice also exhibited the second-set response, while there were fewer enhanced growths of the first inoculum. Now it has been shown that cortisone will cause marked involution of the lymphoid tissues (12), depress the production of isohemagglutinins (33), and inhibit immunological enhancement (27). The present demonstration of the induction of homograft resistance in the presence of cortisone may indicate that resistance is mediated by a variety of cellular types, other than and including the lymphocytes, and that Mitchison's technic of lymph node transfer may reveal only one part of the immunological picture. Whether this is actually so can be decided only after further experimentation, including tests of the capacity of lymphoid tissues from cortisonized animals to passively transfer homograft resistance.

We shall now briefly consider the possibility of "immunological selection" (in the sense in which Hauschka et al. (20) have defined the term) as the basis for enhancement. The plausibility of such an assumption has been considered previously (31) and the reasons for ruling it out have been stated in detail. More recent data bearing against this hypothesis are presented here (29). Enhanced Sarcoma I was subjected to the "second-set" response by being regrafted into C57BL/Ks mice which had been immunized with an inoculum of unenhanced Sarcoma I (taken from A/Ks mice). The enhanced tumors were 2-week-old grafts growing progressively in C57BL/Ks mice which had been passively immunized with C57BL/Ks anti-Sarcoma I serum. (Supporting evidence that these tumors were enhanced comes from the other C57BL/Ks mice in the group, which were not used as tumor donors and went on to die with progressively growing tumors.) The time interval between the immunizing (first) inoculum and the grafting of the enhanced tumor was varied between 1, 7, 14, and 28 days. The enhanced grafts inoculated at the 7-day interval exhibited the typical consequences of a "second-set" response, that is, smaller growth and much more rapid regression than the first graft or no evidence of progressive growth at all. In accord with our previous experience (31), as the time interval between the two graftings was increased (to 14 and 28 days), enhancement of the second graft (the enhanced donor material) now became evident. (In fact, these tumors grew at an astonishingly rapid rate, and most of the hosts died within 4-5 weeks after inoculation of the tumor. The usual mean mortality experience with enhanced Sarcoma I in C57BL/Ks hosts is death at 6-7 weeks.) Clearly then, the enhanced tumors had not been altered in their antigenic
specificity, at least with respect to the antigens involved in both graft destruction and enhancement.

We now pass to a consideration of the hypothesis that enhancement is due to some "physiological" alteration in the tumor, induced by its contact with antiserum, which insures its survival despite the hostile responses of the host. An indication of some such change is the accelerated growth rate that may be exhibited by an enhanced tumor. The markedly accelerated mortality of C57BL/Ks mice receiving enhanced Sarcoma I has been mentioned above. In the same experiment, the stimulated growth was evident also in A/Ks mice (the native strain) which were used as controls for viability of the tumor grafts. In nineteen A/Ks females receiving their grafts from A/Ks donors, the mean survival time was 6.1 weeks (range: 4–8 weeks), while it was 2.9 weeks (range: 2.7–4 weeks) in five A/Ks males receiving the "enhanced" Sarcoma I.

(Casey and Gunn have reported [10] permanently accelerated growth of serial transplants of an indigenous tumor after it had been passed through mice pretreated with killed tumor tissue. There is a difference between our experiments and those of Casey and Gunn—they were using "isologous" grafts derived from tumor "enhanced" in actively immunized mice of the native strain, while we used grafts derived from homografts in passively immunized mice. It is thus not certain that the steps leading to accelerated growth are the same in both instances, particularly since Casey did not try the effects of passively transferred antiserum. However, it is possible that there was sufficient antigenic difference between Casey's tumor—which has a long history of transplantation—and the host strain, so that the grafts were not strictly isologous and immunological enhancement may have been operative. This likewise may be the case in the reports by Shear et al. [48] and Miroff et al. [41] of accelerated growth of a mammary tumor in mice of the native strain and in F1 hybrids when the animals had been pretreated with killed tumor tissue or a lipide extract of the tumor. These investigators also did not try the effects of passively transferred antiserum.)

Gorer (18) has reported an accelerated growth of a leukemia and a sarcoma in the indigenous strain of mice after the tumor cells had been exposed to isoantisera in vitro. We have already mentioned Gorer's finding (15, 16) that a burst of mitotic activity takes place in Sarcoma I cells on the 7th day after inoculation into passively immunized mice. Further evidence in support of the assumption of a "physiological change" comes from experiments (Kaliss, unpublished data) in which small pieces of Sarcoma I were immersed overnight in various normal and immune sera at 0° or 37° C., and also in a tissue culture medium ("ML 192/2," kindly supplied by Dr. Charity Waymouth—see [59]). After removal from the various media, the pieces of tumor were washed several times with Ringer's solution and inoculated subcutaneously in untreated C57BL/Ks hosts. Many of the inocula grew progressively, including those that had been in normal sera and in the tissue culture medium. It is only speculation, but perhaps a parallel can be drawn between this example and the conditions of immunological enhancement, the point in common being injury to the tumor cells with a reaction that is expressed as enhanced (stimulated) growth of the graft.

We have found (unpublished data) that an enhanced tumor (the C57BL/6 carcinoma, E 0771) would grow progressively in a proportion of strain A/Ks mice. This was possible for seven successive transplant generations, but none of the implants survived the eighth transplantation. A similar result was obtained with a line of the strain A tumor Sarcoma I, originally enhanced in C57BL/Ks mice and then serially transplanted in C57BL/Ks mice. The grafts grew progressively for three successive transplant generations but did not survive a fourth transplantation. Grafts from the same tumor all grew progressively in the native (A/Ks) strain. Variations in behavior were observed among different lines of enhanced Sarcoma I; some did not survive the first regrafting into untreated C57BL/Ks hosts, though they did exhibit a marked acceleration in growth rate and reached an unusually large size before regression.

Other investigators (3, 38, 39) have reported experimentally induced alterations in transplantable tumors, though the conditions for producing the changes may be different from that of immunological enhancement. The alteration (an ability now to grow progressively in "foreign" strains of mice) represents a permanent change. Since serological studies were not done in all cases, the nature of the change is not always clear. It may be possible that immunological selection was operative in some instances. Koprowski et al. (39), however, believe that an antigenic change is not involved in their case. Klein and Klein's experiments (38) indicate that the induced changes in specificity observed in their studies cannot be ascribed to antigenic selection, but rather is "adaptive" in nature.

(A recent report of Révész [47] presents a situation which may have a parallel with enhancement. Stimulation of the growth of isologous tu-
mor grafts was observed if the implant consisted of a mixture of viable, untreated tumor cells and killed or damaged x-radiated cells. The stimulation was directly attributable to the presence of the damaged cells (or their products), but the underlying processes are not yet clear. Three possibilities are postulated: [a] specific stimulation by homologous cell products; [b] a “feeder effect,” in which the dead cells release essential nutrients; and [c] stimulation through provoking an inflammatory response and/or vascularization from the host.

Whatever the nature of the change that culminates in enhancement may be, sufficient time as well as opportunity apparently are required for its realization. Stated otherwise, the graft must not be subjected to a second-set reaction, or it will be destroyed before it can traverse the steps toward enhancement, even if enhancing antiserum be supplied by passive transfer. Gorer’s account (15, 16) of the histology of the enhanced tumor describes a distinctive change in the cytology of the graft which first becomes evident about 7 days after its inoculation. This, of course, does not give us information about possible biochemical changes on the path to enhancement, without obvious attendant morphological changes, which may be in progress during this period. The assumption that a change must be taking place follows from our experiments with two successive tumor inoculations in otherwise untreated mice (31), in which it was not uncommon for the first inoculum to resume progressive growth (i.e., become enhanced) after the second graft was inoculated, while the latter graft was rapidly destroyed by the heightened reactivity of the host (Chart 5). It can be postulated that the first graft had had time to undergo a “pre-enhancement” change before the rejection reaction of the host had reached full potency and that the additional stimulus of the antibody later added by passive transfer, or produced as a secondary response to the second tumor inoculation, carried the graft on to full enhancement. The second inoculum, meanwhile, was rapidly destroyed by the already present heightened resistance of the host, before the graft had had time to go through the initial phases of enhancement. The enhanceability of a single inoculum by antiserum injected 7 days after grafting (Chart 2), when the resistance response of the host is already at its full peak, is further support for the assumption that the graft undergoes some kind of “pre-enhancement” change during the period of inception of the host’s immune responses.

It is now necessary to examine in general the manner in which different transplantable tumors may be affected by contact with putatively cytotoxic antisera, because enhancement apparently represents only one part of the possible range of reactions. Comparative studies along these lines have been carried out in greatest detail by Snell and his co-workers (56, 57), and more recently by Gorer and his associates (17, 18) and by Amos and Day (1). Snell, in a recent analysis of the literature (53), has made a three-fold classification of transplantable tumors which can be summarized as follows: (a) tumors that are completely, or almost completely, destroyed by contact with antisera (chiefly characteristic of the leukoses); (b) those that are partly destroyed or temporarily inhibited (tumor B.P.8 is an example); and (c) those that respond almost wholly by enhancement, with little or no destruction of the tumor (Sarcoma I).

It must be emphasized, however, that the classification for any one tumor is determined by the experimental conditions governing the observations. It is conceivable, for example, that a leukemia could be destroyed by antisera under one set of conditions and enhanced under another, if the proper set of requirements for each effect were known. An example is furnished by one of Gorer’s experiments (18) in which a leukemia was exposed to antiserum in vitro. Inocula of large doses of the exposed cells gave accelerated growths in the host, while the smaller cell doses gave inhibited growths. (There is an interesting parallel between these results and the recent report of Révész [47] on his experience with irradiated Erlich ascites tumor cells. A large dose of living cells mixed with lethally damaged irradiated cells gave accelerated growths, but if a small inoculum of viable cells was mixed with the same number of damaged cells, there was a pronounced inhibition of growth.)

Our ignorance of the complexity of these requirements certainly accounts for the bewildering volume of seeming contradictions that appears in the literature on resistance or susceptibility to transplantable tumors. The nature of some of these variables and how they may operate in a specific experimental situation has recently been demonstrated in a series of detailed studies by Snell (55). They serve as an object lesson of the necessity to define precisely the conditions determining the outcome of any experiments in which transplantable tumors are used as research tools.

A few comments are necessary on the matter of terminology. We refer specifically to such usages of convenience as “enhanced mouse,” “actively or passively enhanced mouse,” “enhancing sub-
stance.” These are, in effect, “loaded” terms, and the danger in their use lies in their being taken by the unwary to denote the actual mechanisms involved in enhancement. The initial requirement for enhancement is contact between the graft and circulating antibody, and it is of no concern to the graft whether the antibody is present because of active or passive immunization of the host (which does not mean “active or passive enhancement”). In this context, an “enhancing substance” makes its effect felt in its capacity as an antigen, and the term is open to objection in that it may be misconstrued to suggest that the “substance” is some form of direct “growth promoting stimulant” for the graft.

Enhancement of normal tissue homografts.—We have tried this with skin (in collaboration with Mr. B. F. Bryant), with spleen, and with embryo mince and ovary (in collaboration with Dr. Kathleen P. Hummel), but without success. In contrast to our failures, there are reports of significant prolongations of the survival time of skin grafts in rabbits (5) and mice (4) receiving prior injections of whole blood or various tissues, but the attempt to accomplish this in mice by passive transfer of antiserum has been unsuccessful (P. B. Mevilwar, personal communication). As Dr. Gorser has suggested to us, it is possible that the requirements for a particular antiserum dosage may be even more critical for enhancement of a normal tissue graft than for a tumor. Though Parkes has failed to get prolonged survival of ovary homografts in mice pretreated with tissues (45), he has reported striking success in rats (44, 45). The possible effect of passively transferred antiserum has not been tried, and therefore it is not known whether these results are comparable to immunological enhancement with tumors.

It is obviously important, for both theoretical and practical reasons, to establish whether the reported successes with normal skin and ovary are actually due to immunological enhancement. It is possible that the ability of a tumor graft to become enhanced characterizes a fundamental difference between cancerous and normal tissues. On the other hand, it may be that one road to success in the homografting of normal tissues may lie in discovering the conditions for rendering the tissue adaptable to a new environment, and immunological enhancement may offer one positive method.

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Immunological Enhancement of Tumor Homografts in Mice: A Review

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