PRELIMINARY REPORT ON THE EXPERIMENTAL INDUCTION OF METASTASES FROM A HETERLOGOUS CANCER GRAFT IN MICE*

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In our studies on the inflammatory response to foreign bodies and/or trauma we have utilized cortisone to inhibit normal responses to a variety of traumatic situations such as tuberculosis infection, wounds and irritative agents.\(^1-4\) In continuing these investigations it has been our purpose to elicit information relative to the underlying physio-pathologic disturbances operative in circumventing the animal's normal defenses and therefore resulting in more extensive damage. We extended our studies to "living foreign bodies," i.e., cancer homoiografts.

Previous studies by Kaliss, Snell, Casey have shown that prior injection of mice\(^6-8\) or rabbits\(^7\) with lyophilized cancer or normal tissue from the strain indigenous to the cancer graft used enhanced the growth of the homoiograft in the strain so treated which ordinarily will not grow the graft. Subsequent joint studies between Kaliss and ourselves demonstrated that this phenomenon occurs with antisera to the tissues prepared in either rabbits or mice\(^6,10\) and is also true for grafts of normal spleen tissue in heterologous strains of mice\(^11\) treated with lyophilized normal spleen. This afforded us a convenient test situation in which we postulated that: if the lyophilized tissue injections acted as a systemic trauma thus breaking down the host's ability to handle a second trauma, a live cancer graft; and if we could delay or inhibit such responses by further debilitating influences which specifically interfere with inflammatory responses, i.e., cortisone treatment; then we should be able to further
enlarge the growth of the homoiograft. To this end the following experiments were conducted and are reported here in preliminary form because of the unusually striking results obtained.

Experimental Methods and Findings.—In the following studies the C57BL/6Jax strain of mice were used and the tumor Sarcoma I, an “A” strain tumor which originally arose in A strain mice and was grown by us in A/Cloudman mice.

Tumors were harvested from the A mice under aseptic conditions, lyophilized and resuspended in distilled water on a dry weight basis for injections. The live tumor grafts were fragments of Sarcoma I implanted by trocar in the supra- scapular region. Tumor growths were followed by palpation and by serial sacrifice and autopsy of mice in each group. Cortisone was administered subcutaneously in an aqueous suspension starting 0.5 mg. per mouse daily for 3 days and maintained by a daily dose (except Sundays) of 0.1 mg. per mouse thereafter.

Group I. Seventy mice cortisone maintained, injected with 3 doses 15 mg./mouse each of lyophilized Sarcoma I at 4-day intervals, followed by a live graft implant 1 week later. Cortisone was discontinued in 30 of these mice at the time of tumor graft and the remainder maintained on cortisone.

In both of these groups of mice the homoiograft grew slowly and in many began to become soft and necrotic. Fourteen days after tumor graft several animals exhibited distended abdomens and on sacrifice it was found that the abdomen contained ascitic fluid and extensive intraabdominal sarcomatosis involving mesentery, serosa of intestines, diaphragm, mesenteric nodes, pelvis, retroperitoneal tissues, peri-renal area, right kidney, liver. On subsequent days, the remaining animals showed similar metastases. Subsequent microscopic examination confirmed the gross finding and revealed sarcomata in other organs, e.g., pancreas. The detailed findings and analysis of the data will be reported at a later date when complete serial section studies are made along with data on mice to be sacrificed prior to tumor graft and daily after implantation.

Group II. Control. Twenty-seven mice treated with lyophilized Sarcoma I as above and injected daily with the suspending diluent used for cortisone suspension. Tumor implanted as above. No evidence of metastases was noted. The primary graft was growing progressively in all mice at time of sacrifice. Sacrifices were made at the same time to parallel group I.

Group III. Controls. Thirty mice treated with cortisone only and Sarcoma I implanted as above. Primary tumor was regressing in all mice and at autopsy the primary graft was necrotic and no gross evidence of spread noted.

Group IV and V. Controls. Sarcoma I implanted in 20 C57BL/6Jax and in the indigenous strain, 20 A/Cloudman mice. The graft regressed in all
the C57BL/6Jax and grew progressively in the A/Cloudman strain. No
evidences of abnormalities were found in any of these mice at autopsy.
In another series of studies the above experimental conditions were
duplicated in adrenalectomized mice. At this time the following preliminary
findings are in hand:
1. Adrenalectomized mice treated as group I above do not show any
gross evidence of metastases of the heterologous Sarcoma I.
2. Lyophilized-tissue-treated adrenalectomized mice grow the Sarcoma
I implant whether treated with cortisone or not. If anything, adrena-
ectomy further enhances the Sarcoma I implant.
3. Untreated adrenalectomized mice do not grow the Sarcoma I.
To test the viability of the heterologous metastatic sarcoma fragments
of the tissue were taken from the mesentery of two of the mice at autopsy
and implanted into 6 normals of each of the following strains: C3H/Jax,
C57BL/6Jax, C/Scott, Street, DBA and A/Cloudman. The grafts were
palpable in all six of these strains within 4 days and at 7 days were all larger
than 2 cm. on palpation. In the Street strain the grafts were over 3–4 cm.
All six strains were sacrificed on the 9th day and the tumor and organs
fixed for microscopic histo-pathological study. A duplicate set of the
above strains similarly implanted are currently under observation for ulti-
mate fate of the graft. It appears that the metastatic sarcomata may have
lost their strain specific character. This remains to be established by
more critically controlled experiments. Kaliss¹² is currently observing a
similar change in strain specific character of a homoiograft from lyophil-
ized tissue-enhanced mice in untreated mouse strains to which the tumor is
not indigenous.
A parallel series of studies is under way in which wounding is used as a
means of traumatic stimulus in addition to an homologous cancer graft.
In these studies as in the above, it is intended to elicit information concern-
ing the influence of the state of “reacting to trauma” on the graft. The
preliminary experiments involve two situations: a rapid- and a slow-
growing implanted tumor.
The rapidly-growing tumor is the Sarcoma S621 in C/Scott mice, the
slow-growing tumor is the BW1898 in C57BL/6Jax mice. Each test set-up
is comprised of 3 groups: control implant in normal mice; graft implant
intramuscularly and tangent to a surgically induced wound on the back
involving skin, subcutaneous fascia and muscle, the implant made 4 hours
after wounding and the wound allowed to heal; graft implant intramuscu-
larly and tangent to a wound as above, but the wound surgically opened
daily in order to keep a situation of continuous “granulating wound.”
The Sarcoma S621 in the control (not wounded) C/Scott mice grew
progressively and all animals were dead with large tumors which had
broken through the muscle wall and were growing intraabdominally in 11
to 19 days. This is average for this tumor/strain. The wounded C/Scott mice in whom the wounds were permitted to heal differed slightly from the controls: the graft grew more slowly and in $\frac{1}{2}$ the animals was not palpable until the 11th–15th day; deaths with tumor occurred between the 19th and 26th day. The mice in whom the wounds were surgically opened daily were considerably slower in growth; the first deaths with small tumors occurring on the 20th day; the remaining 10 mice in this group were sacrificed on that day for comparative autopsy. It was found in this latter group that the tumor was smaller, well encapsulated, necrotic and did not break through the dorsal muscle wall into the thorax and abdomen. The tumors in the control mice had broken through and extended growth into the abdomen and showed moderate central necrosis. It appears that the maintenance of an active inflammatory response in the local tumor site acts to “contain” an homologous cancer graft which grows more slowly than in normal mice.

The experiments on the slow-growing graft are still in progress. It appears at this writing that the same results are being obtained.

A recent report of cortisone-induced metastases of an homologous cancer graft in mice adds further to the data we are obtaining in support of our hypothesized physio-pathologic mechanism of action. These latter investigators, however, attribute their findings to a “tumorigenic” action of the cortisone despite their own finding that cortisone delayed and arrested the cancer implant. In addition they used a strain of mice (C3H) known to possess the mammary milk factor with a mammary adenocarcinoma and this may play a role in their contradictory findings. It is beyond doubt, however, that growth of intraabdominal sarcomata was induced and it remains now to establish the mechanism by further experimentation.

It is interesting that we were able to predict our results based on our tentative hypothesis of mechanism knowing full well that a heterologous cancer graft has never been made to metastasize.

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10 Casey, A. E., Ibid., 31, 663–665 (1934).
AN INVARIANT FORCE FUNCTION IN MUSCLE ACTIVITY

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When a single nerve impulse reaches a neuromuscular junction it initiates a chain of events culminating in the characteristic twitch response of the muscle fiber. If the fiber length is fixed during activity the tension rises to a maximum and declines gradually to the resting level. The so-called isometric twitch is a basic all or none increment of activity in the sense that its magnitude and time course are independent of the nature of the stimulus. All suprathreshold electric current stimuli elicit more or less identical twitches regardless of current duration or rate of change of current. Any continued maintenance of actively developed tension is possible under normal conditions only in the event of repetitive stimulation of the fiber. A progressive summation of staggered twitches then suffices to maintain tension above the resting level. A fundamental point to emphasize is that muscle activity is manifest either as a twitch or as a sum of twitches.

However, the magnitude of the isometric twitch can be altered by changing certain variables. As the length of the fiber is increased, the maximum change in tension during a twitch is reduced, but the time parameters of tension change are unaltered in an unfatigued fiber. This at once suggests that some invariant process may underlie each isometric twitch regardless of extent to which the tension may change.

However, isotonic activity of muscle involves further complications. If a muscle shortens against a constant load the time course of the corresponding twitch is not completely independent of the load. It is the purpose of this investigation to disclose a unique all-or-none time function which is invariant for all muscle twitches carried out isotonically. By relating the isotonic peak twitch deflection to the derivative of length with respect to tension, it can be shown that a constant force is generated within the muscle at the time of the twitch deflection maximum regardless of existing tension or length. In so far as the time course of the isotonic