data base now exists for the design of the gasification section of a large pilot plant. The recent demonstration run was a major milestone in the CCG development program because it provided confidence in our ability to achieve commercial operating conditions in the PDU.

Concluding Remarks

Research and development on catalytic coal gasification for methane production is proceeding well. The operation of a process development unit at 900 kg/day has demonstrated operability and identified a number of problems for which solutions have been found. An essential aspect of CCG development is the close coupling between basic, bench-scale, and PDU research and engineering studies. The next major step is the design, construction, and operation of a large pilot plant (90,000 kg/day) in the middle 1980's. Success in future research and development could bring CCG to commercial readiness in the later 1980's.

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15. We acknowledge and greatly appreciate the efforts of the large group of scientists, engineers, technicians, and support personnel who have contributed to the success of the CCG program. We particularly thank C. A. Euker and N. C. Nahas, who headed the CCG Process Development Section at Baytown, Texas, during the formative periods of the program. While Exxon funding was used to initiate CCG research and to construct the PDU, funding from the Department of Energy and the Gas Research Institute carried the program through the predevelopment and early development phases. Their support and encouragement are gratefully acknowledged.

Regional Differences in the Growth of Normal and Neoplastic Cells

Robert Auerbach and Wanda Auerbach

In 1936, when J. M. Twort and C. C. Twort painted mice with a synthetic tar dissolved in chloroform, they discovered that the onset of visible tumors was markedly delayed in those mice treated with the carcinogen more posteriorly in the sacral region compared to those treated anteriorly in the scapular region (*1*). This was not a casual observation, because these investigators in the course of their many studies reported on some 15,000 tumors induced by a wide variety of carcinogens administered to over 100,000 mice. Even the particular experiments cited, on the effects of synthetic tar, involved several hundred tumors, and the records included tumor onset, total incidence over time, and the extent of malignancy. The study was convincing, the results were striking, and there was no doubt that the carcinogen was more effective in the anterior than in the posterior site. Yet the work attracted little attention and was seemingly forgotten.

In 1973 Vaage published a description of techniques used in tumor transplantation (*2*). He mentioned that there are significant differences in the growth of tumor cells inoculated anteriorly compared to those injected posteriorly under otherwise identical conditions. He recognized the importance of precision in the development of injection protocols, and, like Twort and Twort, made a strong plea for consistency in the application of experimental procedures to cancer research. But the discussion of anteroposterior differences was brief and his comments were not generally noticed.

In 1975 Kobayashi (*3*), working at a marine laboratory in Japan, compared the response of the mouse to experimental wounding of the dorsal skin at various levels of the trunk. He observed that there was a higher mitotic index in the epidermis surrounding wounds made anteriorly than around wounds made more posteriorly. But the report was an isolated one, not placed in the perspective of tumor growth, and received little subsequent discussion.

Our own studies came about because of seemingly capricious results obtained in the course of investigating the response of transplanted tumor cells to immune regulation (*4–6*). As we puzzled over the data we eventually discovered that regional differences due to location of the tumor inoculum—even a few millimeters distance within the trunk skin—were of such major importance that they were obscuring any possible differential between control and experimental groups. In trying to gain understanding of this phenomenon we searched the scientific literature. We found here and there anecdotal or modestly documented observations, often incidental to the work being presented. Occasionally we found, sometimes by serendipity alone, substantive works such as those cited above. These works, however, offered no links to the earlier literature and were therefore difficult to place in any historical context. We expanded both our literature search and our own experiments to include normal tissue transplants, drug efficacy, and a variety of physiological parameters ranging from blood flow to the aging process. An underlying pattern of regional influences began to appear.

Even if the various studies of anteroposterior differences in carcinogenesis,

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growth of transplanted tumors, wound healing, immune responsiveness, and blood flow were to bear no relation to each other they should be rescued from obscurity because of their importance to the standardization of research protocols. Experiments designed to compare carcinogenic potential and to examine tumor progression use hundreds of thousands of animals each year. Many experiments involve the systematic administration of drugs to test their action on physiological processes, to measure

Summary. This article provides a review of the literature on anteroposterior differences in the growth and functional expression of normal and neoplastic cells. Diverse studies in immunology, wound healing, carcinogenesis, transplantation biology, and developmental biology are brought together to emphasize the practical implications of regional differences for research protocols in all these areas of research. The more fundamental question of mechanisms is approached by consideration of variations in the vascular supply, pattern of nervous system development, expression of temperature differentials, and the establishment of metabolic gradients during development.

their therapeutic effectiveness, or to monitor their adverse effects. Transplantation procedures are regularly utilized for research in endocrinology, surgery, immunology, and developmental biology. The conclusions drawn from data collected from these many types of investigations may be unreliable if underlying regional growth differences are not taken into account.

The purpose of this review is twofold: to focus attention on the practical importance of recognizing regional differences as a source of major experimental variability; and at the same time to arouse a wider interest in the basic biological processes underlying the expression of regional differences in the growth, differentiation, and functional activity of normal and tumor cells.

Response to Carcinogens

Prehn and Karnik (*7*) reported recently that there were marked differences in the effect of methylcholanthrene pellets implanted subcutaneously into the axillary or inguinal region of the mouse (Fig. 1a). Except at the lowest marginally effective doses of carcinogen, they found that when four simultaneous implants were made, two anteriorly and two posteriorly, the first tumor to arise was almost always (> 90 percent) an anterior one. The results were striking and it seemed remarkable that so little background information could be available, given the importance of the observation. Indeed, in spite of the extensive work on carcinogenesis carried out in mice this appears to be the first systematic analysis other than the studies of Twort and Twort (*1*) published 23 years earlier.

Information on regional differences in response to carcinogens has also been obtained for rats, but again the reports are scattered ones and the implications from these studies have also been left unexplored. Nothdurft (*8*), in a brief note, reported that sarcoma induction in rats in the middorsal region exhibits anteroposterior differentials, tumors arising more frequently anteriorly than posteriorly (Fig. 1b). Ventrally, the pattern was not distinct. A more extensive study was subsequently carried out by Ott (*9*) who demonstrated axial differences in the induction of tumors in Wistar rats given polypropylene implants in the various portions of the trunk (Fig. 1b). While in principle this work confirmed the report of Nothdurft, Ott found that the regional differences, although apparent in Wistar strain rats, did not occur in line E3 rats. The basis for the strain differences is not known.

Torgersen (*10*) has reported further on tumor production in rats (Fig. 1c). In his work on dimethylbenz[*a*]anthracene-induced mammary tumors he showed that there is a more than 2:1 preference for the appearance of thoracic mammary tumors over abdominal ones. Almost identical ratios were reported by Gullino *et al.* (*11*) who studied mammary tumor formation in response to nitrosomethylurea, and by Shellabarger *et al.* (*12*) who observed mammary tumor formation after the administration of either dimethylbenz[*a*]anthracene or procarbazine.

Growth of Transplanted Tumors

When we inoculated tumor cells intradermally into more cranial regions of the lateral trunk of adult, syngeneic mice, we observed that these cells showed strikingly greater growth and development than did similar cells injected more caudally (*4*) (Fig. 2). We found this to be true for every tumor type tested: S180 sarcoma, B16 melanoma, OTT 6050 teratocarcinoma, NS-1 myeloma, and a variety of lymphoblastoid T-cell lymphomas. The effects were seen equally in irradiated and unirradiated animals, in animals lacking a thymus (*nu/nu*), in males as well as females, and in various mouse strains. When large numbers of tumor cells were inoculated the tumor injected anteriorly developed sooner, grew to four times the size of comparable tumors injected posteriorly, and led to a more rapid death of the host animals (Fig. 1d). When lower cell numbers were used we found that a dose of cells capable of killing half the animals after injection into the thoracic lateral trunk skin resulted in the death of only about 25 percent of animals inoculated at the lumbar level (Fig. 1e).

Our first assumption was that we were dealing with anteroposterior differentials in skin architecture. However, when we used subcutaneous inoculations for the tests of tumor growth, the regional differences were every bit as marked (Fig. 1f). Once again a variety of tumors was used and we found that the onset of palpability, the extent of growth, and the percentage mortality at limiting dilutions reflected anterior-posterior regional differences (*4*).

Even more surprising were the results we obtained with tumor cell inoculations made intraperitoneally (*13*) (Fig. 1h). Using identical procedures for intraperitoneal injection in which we varied only the angle in which the syringe was pointed after midventral insertion, we observed that C755 mammary tumor cells injected with the needle pointed anteriorly led to twice the rate of tumor mortality obtained when identical cell numbers were injected with the needle pointed posteriorly. While at first this seemed hard to accept, we found that the adhesion of inoculated cells reflected the angle of insertion; that is, more cells adhered to the peritoneal lining in the area where the cells were deposited than in more distant regions.

A more precise interpretation of the intraperitoneal results was made possible by comparing the growth and mortality of mice inoculated with two types of S180 sarcoma cells, derived from a solid tumor or obtained from one adapted to growth in suspension (ascites) form. When S180 cells derived from the solid tumor were inoculated intraperitoneally they behaved similarly to the C755 mammary tumor: more than twice as many animals died when the S180 cells were inoculated in an anterior direction compared to when they were inoculated in a posterior direction. In contrast, no re-

gional differences were seen when suspension-adapted S180 cells were inoculated. We concluded that regional differences can be observed intraperitoneally only when cell adhesion is required for growth. Such adhesion permits the manifestation of factors associated with the local tissue environment. Cells adapted to independent growth would not be subject to such regulatory, regionally influenced differences.

Having established a clear anteroposterior differential in the growth of certain tumors we attempted to determine more precisely the type of local differences that might exist in the body. To date the main extension of the regional map has been limited to the intradermal inoculation sites. Using the C755 mammary tumor we observed that there were both anteroposterior and dorsoventral differences and that tumor growth within the trunk skin was most rapid in the lateral and dorsal thoracic region, slowest in the dorsolateral lumbar region, and intermediate midventrally (*6*).

Of earlier studies note should be taken of the observations of Hewitt and his coworkers (*14*) who reported that tumors transplanted anteriorly (subcutaneously) were more likely to grow ($P < .01$) than tumors grafted posteriorly. These workers also noted, without further comment, that tumor cell inocula growing anteriorly grew to greater size than did an equivalent number of cells inoculated more posteriorly. They attributed differential incidence to vagaries of the inoculator rather than the inoculum, however, and did not further question the basis for the observed regionality. The work of Vaage and his co-workers (*2, 15*) on regional differences in the growth of inoculated tumor cells has already been mentioned.

Within this past year a greater awareness of the importance of regional differences in tumor growth has been reflected in general discussions of research protocols (*16*).

Incidence and Growth of Spontaneous Tumors

Since virtually all tumors are tissue or organ specific, both in terms of origins and in the type of metastatic behavior, one is hard put to find tumor sites that afford an opportunity to examine the effect of regional, topographical differences as distinct etiological entities. Perhaps the most well-studied tumors in this respect are the mouse mammary tumors, where the incidence of neoplasms arising in the more anterior as opposed to posterior mammae has been documented. In

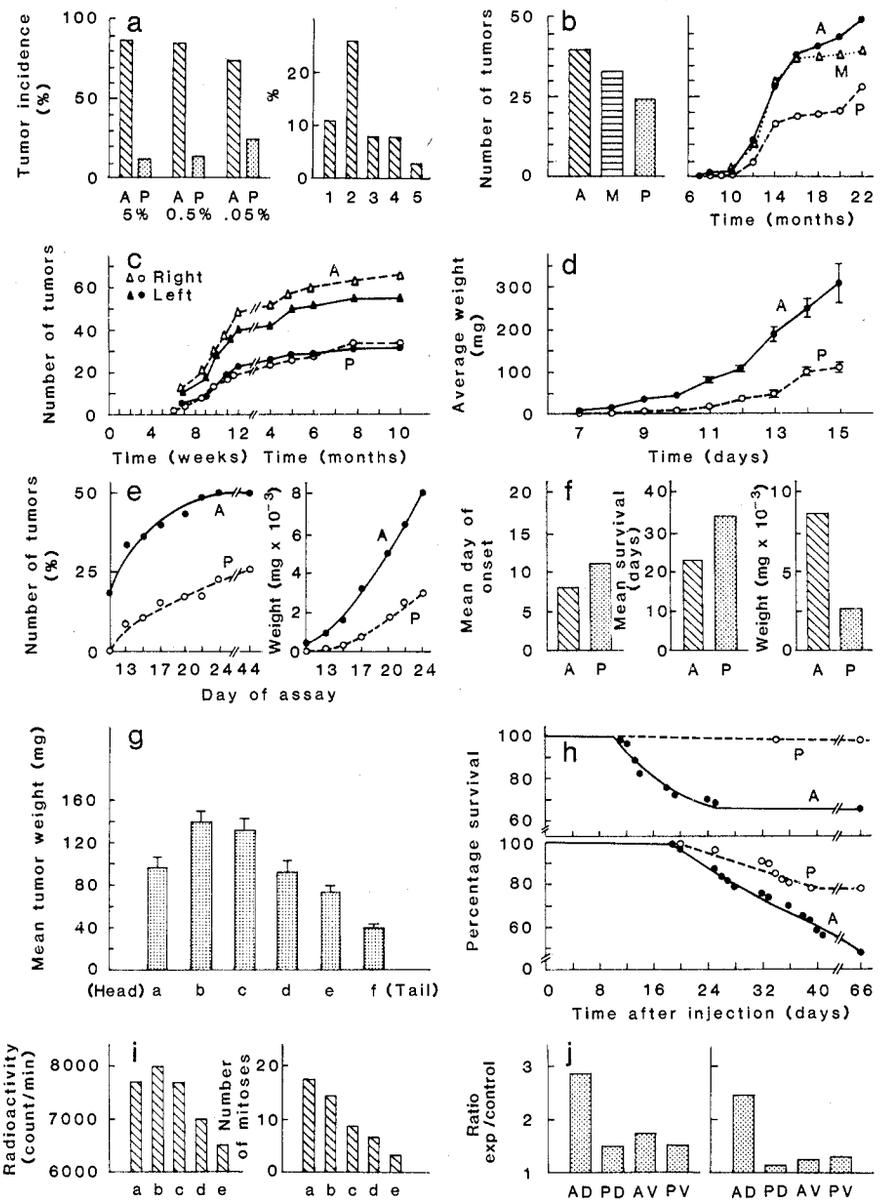

Fig. 1. Regional differences in (a) spontaneous tumor incidence and (a to c) response to carcinogens; (d to h) in growth of transplanted tumors; (i) in epidermal mitosis after injury; and (j) in the systemic response to local sensitization. (a) (Left) Differential susceptibility of axilla (*A*, anterior) and groin (*P*, posterior) to induction of subcutaneous sarcoma by 3-methylcholanthrene administered to mice (*7*). (Right) Spontaneous occurrence of mammary tumors in mice. The numbers 1 to 5 refer to the five pairs of mammae, 1 being the most anterior (*19*). (b) (Left) Number of tumors induced anteriorly (*A*), in the midregion (*M*), or posteriorly (*P*) after the introduction of polypropylene pellets into the mouse flank [data from (8) and (9), combined]. (Right) Number of tumors appearing at various times after initiation of treatment (*9*). (c) Cumulative number of tumors observed in thoracic (*A*, anterior) and abdominal (*P*, posterior) mammary glands after intravenous administration of dimethylbenz[*a*]anthracene (*10*). (d) Tumor weights obtained at various times after transfer of C755 mammary tumor cells into anterior (*A*) or posterior (*P*) intradermal sites in the mouse (*4*). (e) (Left) Percentage mortality and (right) tumor weight after intradermal transfer of C755 tumor cells (*4*). (f) (Left) Mean day of onset, (center) length of survival, and (right) tumor weight after subcutaneous transfer of tumor cells (*4*). (g) Gradient-like pattern of tumor growth obtained after intradermal inoculation of tumor cells at various points along the middorsal axis of the mouse trunk (*6*). (h) Survival curves obtained after an intraperitoneal injection of (top) 2×10^4 cells of an S180 solid tumor and (bottom) 10^4 cells of a C755 mammary tumor (*13*). (i) Cell division measured in dorsal epidermis after (left) plucking of hair (*30*) or (right) wounding of the skin (*3*). (j) Delayed hypersensitivity responses to trinitrophenylated cells after sensitization anteriorly (*A*), or posteriorly (*P*), dorsally (*D*), or ventrally (*V*) in the mouse skin (*65*), as determined by (left) ear pinna thickness (the data show the ratio of thickness of the antigen-injected pinna to that of the saline-injected pinna), and (right) the accumulation of ^{125}I-labeled iododeoxyuridine (the data show the ratio of labeled cells in the experimental pinna to the number in the control pinna) (*65*).

1952, Pullinger (*17*) observed that anterior mammae of mice are more likely to develop tumor nodules than posterior ones. Prehn and his colleagues (*18*) extended these studies, but while confirming Pullinger's observations, determined that site preferences are not seen in those mouse strains in which the mammary tumor virus is present. Squartini (*19*) obtained similar results, finding anteroposterior differences in mammary tumor incidence in BALB/c and RIII strains (aviral) but not in the C3H, virus-carrying strain. In one study (*20*) regional preferences for mammary tumor formation in mice were not found, but the viral status of the research animals used was not known. The regional differences could not be correlated with histological differences of the mammae, with their relative size, or with differences in lactation.

In contrast, the distribution of mammary tumors in dogs appears to be the reverse. Cameron and Faulkin (*21*) found that both hyperplastic and inflammatory nodules are more frequent posteriorly, confirming the extensive studies of Moulton *et al.* (*22*). A detailed study of Warner (*23*) provides additional evidence of spontaneous mammary tumors occurring more frequently in the posterior than anterior mammae of dogs. Cotchin (*24*) had earlier observed similar differences, although in a low number of animals, and had suggested that these differences were due to the fact that in dogs the posterior mammary glands are larger; but precise measurements of cell number or of comparative histology have not been carried out. Huggins and Moulder (*25*) attributed the differences to the fact that abdominal mammary glands retain secretory activity for a longer time.

Growth of Transplanted Normal Tissues

One's first inclination is to look for some unique property of tumors that might account for their preferential growth and development at one or another point along the trunk axis, some tumor-specific factor that recognizes locational cues. However, one can also take the view that the results might not be attributable to the nature of tumor cells, but rather might represent a more general property of host factors, unequally yet predictably distributed, that would also be manifested when studying normal cells and tissues. The controversy between "soil" and "seed" in regulating tumor growth goes back well into the 19th century (*26*) and, much like the

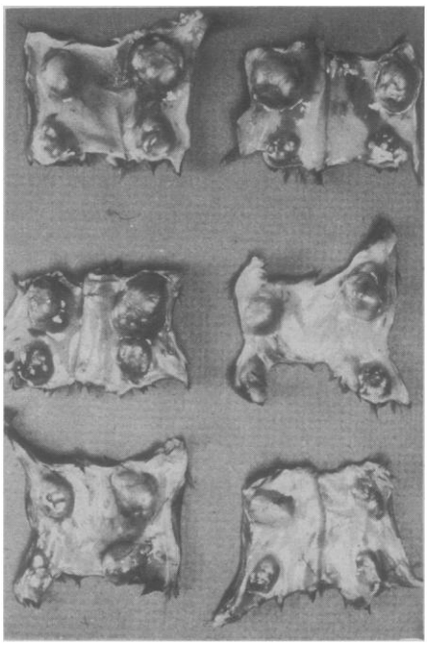

Fig. 2. Regional differences in the growth of C755 mammary carcinoma cells injected intradermally into adult C57BL/6 (syngeneic) mice. One complete set of six animals is shown to indicate range of growth differences. The anterior skin sites are at the top of each animal (*4*).

generic arguments concerning environment versus heredity, has by no means been resolved simply by the passage of time. It is interesting indeed that the soil versus seed discussions appear to have been restricted to analyses of malignancies and have not extended to an analysis of normal growth processes except, perhaps, in the much narrower context of the *Entwicklungsmechanik* studies of the experimental embryologist.

To test systematically whether the observed regional effects might apply to normal tissue growth as well, we have now analyzed several different experimental situations. Our first approach was to analyze the behavior of skin grafts between fully histocompatible mice differing only in the presence of a pigment marker (albino or black) to facilitate observations. When we compared the growth of anteriorly placed grafts with those placed more posteriorly, always along the middorsal line, we found that grafts placed more anteriorly healed more rapidly and ultimately were maintained as larger grafts than were the ones placed more posteriorly (*27*) (Fig. 3).

We next examined the ability of thymus and spleen grafts, obtained from neonatal syngeneic donor mice, to grow at anterior or posterior sites on the mouse trunk following subcutaneous implantation (*28*). We compared growth of thymus grafts in normal adult hosts and we studied spleen grafts placed either in normal or splenectomized recipients. We also compared animals carrying grafts only anteriorly or posteriorly with animals with multiple grafts placed anteriorly as well as posteriorly in the same host. In all instances, both thymus and spleen grafts grew more rapidly anteriorly than they did posteriorly (*28*).

These experiments provide some additional insight into the nature of host regulation of subcutaneously implanted organ grafts. Many years ago, Metcalf (*29*) studied the behavior of multiple thymus and spleen grafts. He observed that thymus grafts grew equally well whether or not a host thymus was present, and that it did not matter whether one or many grafts were present in the same animal. However, the growth of splenic grafts was materially affected by the presence of a host spleen and multiple splenic grafts grew less well than did single implants, that is, the growth of splenic grafts was influenced systemically by the total splenic mass. Metcalf's studies did not consider regional growth differences. In our experiments we confirmed that the presence of the host spleen depressed spleen graft growth. In addition, we showed that anterior and posterior growth differentials were present both in splenectomized and in normal animals, and were apparent both when grafts were compared growing in the same animal and when grafts placed anteriorly in one animal were compared to grafts placed posteriorly in different hosts. The local host regulation of growth of the implanted transplant preceded any systemic influence, the latter becoming apparent only after the transplants had become well established and vascularized.

Topographical Aspects of Wound Healing

The work of Kobayashi, already cited, demonstrated that when comparable wounds were made at various levels of the middorsal skin of mice the rate of subsequent epidermal mitosis, measured by the incorporation of tritiated thymidine, was higher anteriorly than it was caudally (*3*) (Fig. 1i). These results could have been anticipated from the findings of Potten *et al.* (*30*) that the mitotic index in the epidermis observed after the plucking of hairs shows an anteroposterior gradient pattern quite similar to that seen in tumor transplant experiments (Fig. 1, g and i), with a peak in the thoracic region. According to Thévenet and Sengel (*31*), cell migratory activity is also greater anteriorly than posteriorly.

Our own results with skin transplantation resulted no doubt in part from such host epidermal effects subsequent to suturing of the graft on the denuded graft bed, although it is unlikely that the differential rate of epidermal mitosis was sufficient to explain the extent of the observed regional differences. Rather, the results of Kobayashi suggest that the conditions for DNA synthesis and mitosis are more favorable anteriorly than posteriorly, a suggestion which is borne out by our observations (*28*) that the initiation of DNA synthesis is more rapid in tumor cells inoculated intradermally into anterior sites than it is in tumor cells inoculated posteriorly.

Kullander and Olsson (*32*) studied another aspect of wound healing. These investigators made experimental wounds in the backs of rats and subsequently examined the tensile strength of the wound closure 7 days after experimental insult. Although the primary purpose of the experiment was to examine wound healing differentials during pregnancy, they observed incidentally that the strength of pull needed to reopen a wound anteriorly was greater than what was needed posteriorly, that is, the extent of wound healing was greater in a more cranial location than further down the trunk. Only in the "most caudal" segment (presumably along the tail) was there deviation from the strict anteroposterior gradient of healing. Kullander and Olsson's comment that "it is important when studying wound healing to compare cutaneous wounds made in exactly corresponding positions" long precedes the observations of Kobayashi and our own studies of transplanted skin. Their suggestion that differences in the vascularity of different regions of the skin may play a role in evoking the results observed will be examined more critically below.

A complete review of the many facets of wound healing that have peripheral or potential relevance to regional effects is not feasible, and reference is made instead to the exhaustive discussion by Arey (*33*) of the overall physiology underlying the wound healing process. An extensive consideration of regional aspects of skin growth and repair is seen in the work of Kiljunen (*34*) who describes, for example, the mitotic activity of epidermis in different parts of the body both in normal animals and in animals stimulated with tumor cells. Although Kiljunen addresses dorsal and ventral differences, his own studies involved the pooling of data from various dorsal skin sites, so that anteroposterior differences were obscured by the treatment of the

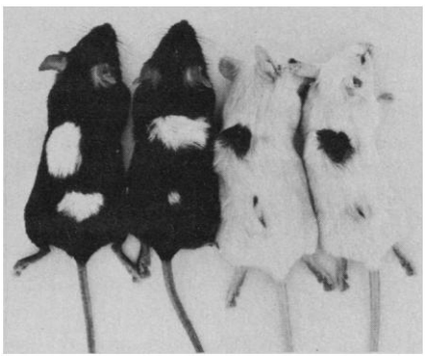

Fig. 3. Reciprocal skin grafts between C57BL/6J (black) and C57BL/6$^{c^2}$J (white) histocompatible mice. Grafts placed anteriorly and posteriorly were initially identical in size (*27*).

results. Finally, one needs to be aware of the detailed studies of Iversen and his associates on epidermal wound chalones; these are described along with a review of the literature in a recent chapter by Iversen (*35*).

Axial Patterns in Vascularity

That a common element underlying the observed differences in tumor growth, in the behavior of organ grafts, and in wound healing may be the vascular system is supported by both direct and indirect observations. In using microprobes to examine skin temperatures along the mouse trunk we observed that at room temperatures there was a differential of about 0.5°C, such that posterior skin was cooler than anterior skin. The differential was greater (0.75°C) when animals were kept at cooler ambient temperatures (15°C) and virtually extinguished when animals were kept at warmer temperatures (33°C). Since skin temperatures generally are most influenced by the local microcirculation we considered this to represent indirect evidence for microcirculatory differences (*5*). (We point out, however, that change of the external temperatures did not materially influence the regional growth pattern of tumors transplanted intradermally.) These observations were further strengthened by the use of thermosensitive color crystals that demonstrated that there was both an anteroposterior and a dorsoventral gradient pattern of temperature ranges; this pattern correlated remarkably well with the observed gradient effect on tumor growth (*6, 28*).

The mutant mouse himalayan, a variant of albino ($c^h c^h$), provides a natural confirmation for these observations. The pigment pattern expressed in these mice (*36*) shows a differential expression of melanin, reflecting anteroposterior variations in temperature. By controlling the ambient temperature in which mice are kept after the plucking of hairs it has been possible to characterize more precisely the gradient pattern of skin temperatures, seen in the coloration of the newly emerging hairs (*37*).

A more direct measurement of relative blood flow was carried out by administering radioactively labeled microspheres into the arterial circulation of mice (*5*). To our surprise, we found no regional differences within the range of sensitivity of the microsphere methodology: the distribution of microspheres was such that there was an anterior/posterior ratio of blood flow of 0.99. This finding, combined with indirect evidence from studies of the rate of clearance of locally administered sodium chromate, led us to conclude that in the resting animal there were no major differences in blood flow to the various parts of the trunk skin.

That conclusion, it now appears, may well have been incorrect, being based on a method not sensitive enough to detect blood flow differences of less than about 10 percent, given the number of animals used in these experiments. A variety of studies now indicates that local blood flow differences do exist in the skin and that these are suggestive of an anteroposterior gradient. For example, Jirtle *et al.* (*38*) describe regional differences in the flow of blood in the skin of unanesthetized rats with a higher capillary blood flow anteriorly than posteriorly. They point out, moreover, that blood flow measurements in anesthetized animals are misleading because anesthesia itself causes major alterations in the pattern of blood distribution. A variety of surgical studies with the use of skin flaps in dogs points to similar blood flow differences in this species as well (*39*).

Most important, however, are studies in which the test animal is examined after stimulation of the vascular system, be it by wounding, by administration of inflammatory agents, or by injection of tumor cells or fragments. For example, Ottaway and Parrott (*40*) describe changes in regional blood flow during immune responses; Silver (*41*) observes changes induced by hypoxia and ionic alterations; noradrenalin effects are documented by Mattsson *et al.* (*42*); and age-related changes in local blood flow patterns are described by Tsuchida (*43*). Of direct relevance is the work of Minasian (*44*) carried out in association with A. J. S. Davies. Minasian measured blood flow in areas of skin near tumor grafts and observed that the skin overlying the pectoral mammary glands of mice

had a higher fractional distribution of cardiac output than did the skin overlying the inguinal mammary gland; that dorsal skin blood flow inguinally was greater than ventral blood flow; and that skin near tumors had greater blood flow than skin from the equivalent contralateral regions not adjacent to tumors. Earlier studies of Argyris (*45*) also dealt with the relation between tumors and adjacent skin functions.

We have recently used biological assays for regional blood flow that could amplify what might otherwise be differences too small to be detected. One of these involved the intradermal administration of pentobarbital sodium. Comparing the efficacy of injections made in the thoracic as opposed to the lumbar lateral trunk region, we observed that mice injected anteriorly lost consciousness more rapidly than mice injected posteriorly, and that the effective lethal dose was lower for the former than for the latter. Since pentobarbital sodium acts by entering the circulation, the results suggest that, in contrast to our cruder measurements based on radioactive clearance of sodium chromate, there were indeed differences in vascular clearance between the two test sites. A second assay involved the intradermal administration of fluorescein, with subsequent measurement of the appearance of fluorescence in the urinary papilla. Once again the experiment demonstrated that anterior administration of the drug resulted in a more rapid introduction of the tracer material into the bloodstream than did comparable administration more posteriorly.

Anteroposterior Differences in the Nervous System

To the embryologist, developmental gradients almost immediately suggest nervous system development. An anteroposterior pattern of neural tube closure, initiated in the thoracic region and extending posteriorly in a zipper-like fashion as development proceeds, is a most direct reflection of the primary gradient of axial development. Aside from this temporal manifestation, there is a pattern of development of dorsal root ganglia, of sympathetic ganglia, and of neural crest that is characterized by a generally decreasing size so that each segment of the trunk, as it is laid down, is a slightly smaller replica of the preceding one. This is not to say that the differentials so obviously demonstrable throughout ontogeny necessarily remain throughout life—this question has generally not been asked.

In considering the functional role of the nervous system in relation to regional differences it is immediately evident that differentials in neural development lead to vascular differences that might be maintained throughout adult life. Folkow (*46*) and Abramson (*47*) have reviewed the many facets of nervous control of blood vessels and a full discussion of these goes beyond the scope of this article. Of particular interest is the concept advanced by Smith and Wolpert (*48*) that neural stimulation is essential for vasculogenesis accompanying regeneration of limbs in amphibians. This suggestion, admittedly speculative at the time, has been supported by the finding that aneurogenic limbs that normally cannot regenerate will do so if given fibroblast growth factor (FGF), a known stimulator of endothelial cell replication (*49*). In this instance, the FGF apparently can substitute for the stimulus normally provided by the peripheral nerves. Regional complexities of the nervous system that are manifested by differentials in regeneration and neural sprouting have been reported (*50*).

There are several other gradient-type phenomena associated with the nervous system that should be mentioned, even though at the moment there are no direct links between these phenomena and the observed regional growth differences. For example, Ostman *et al.* (*51*) report that there may be such differences in the balance between α- and β-adrenergic receptor responses. Haddow's group (*52*) many years ago showed that anteroposterior as well as dorsoventral differences that can be observed in the coat color of mice injected with flavin are correlated with regional differences in the sympathetic nervous system. A comparable gradient is seen in the activity of gut-associated enzymes along the entire length of the small intestine (*53*). Suzuki *et al.* (*54*) have shown a gradient-like distribution of Lafora-like bodies in the spinal cord of various mammals (increasing with distance from cervical to lumbosacral region). And one may even recall the early descriptions of bioelectric gradients and their association both with the developing nervous system and the subsequent elaboration of blood vessels (*55*)

Developmental Concepts

Throughout this discussion we have been drawn to the concept that anteroposterior and dorsoventral differences exist in the regulation of tumor growth, graft maintenance, wound healing, and various physiological activities, and that the basis for these differentials may in some way be related to the kind of developmental gradients so frequently associated with embryonic development. Kobayashi (*3*) tried to explain his observations on epidermal mitotic rate differences through invoking such gradients; Prehn and Karnik (*7*) made similar reference to gradient concepts to explain differential rates of carcinogenesis; and we, in our own work (*6*) drew parallels between the behavior of transplanted tumor cells and basic developmental gradients.

Metabolic gradients were first discussed more than half a century ago when Child (*56*) proposed an anteroposterior gradient concept as "the" basic principle underlying axial differentiation. A second, dorsoventral gradient provided the necessary additional variable for permitting, on a theoretical basis, the precise identification of all the coordinates of a developing system (*57*). The gradient concepts at the same time were a practical means of describing the many observations resulting from experimental manipulation of developing embryos. More recently, there have been renewed efforts to use coordinate systems to explain polarity of developing organs and to predict the outcome of transplantation experiments with both vertebrate and invertebrate embryos (*58*). However, in spite of the value of theoretical approaches for organizing existing information and for predicting the outcome of particular experimental operations, the search for a biological or biochemical basis for these gradients has been unsuccessful. Only the first, the anteroposterior gradient, has been associated with one functional parameter, namely, respiration, and the suggestion of Child (*56*) that the primary gradient is a respiratory one, reflected in oxygen availability and consumption, is still tenable.

It is thus interesting that in our analysis we return repeatedly to consider the vascular system as the primary mediator of the observed regional responses. The role of blood vessels as regulators of tumor growth in particular, already emphasized in the mid-19th century (*59*), has been examined most critically in the recent studies of Folkman and his collaborators (*60*); these workers concentrated their attention not only on the induction of blood vessels by tumors but quite directly on the effect of reduced vasculature on the regression and regulation of tumor growth. Thus they have shown not only that avascular tumor nodules fail to grow beyond a limited size, but they have demonstrated as well that in the presence of inhibitors of angiogenesis tumors fail to develop and may even

regress. Other regionally influenced processes, such as graft maintenance, establishment, and growth, as well as various facets of immune reactions, are also directly influenced by the vascular supply. The previously described temperature differentials must certainly be manifestations of quantitative differences in the extent of the local microvasculature.

Most compelling is the association of the primary embryonic gradient with the establishment of the nervous system. Vascular development is under the direct control of neural influences. If the pattern of vascular arborization follows from the expansion of the peripheral nervous system, then a continuum can be imagined that begins with basic metabolic gradients which become permanently fixed through the sequential development of neural differentials and lead to permanent anteroposterior differences in the mature vascular system.

Nowhere, perhaps, can the connection between the nervous system, vascular pattern, and developmental gradients be seen more clearly than in pigment cell formation and establishment of pattern as manifested during embryogenesis and as it changes during aging and following experimental insult (*57*). Neural crest cell migration, leading to the peripheralization of melanocytes, occurs in an anteroposterior progression, with cells moving also from their dorsal origin toward the ventral midline. The appearance of hair pigmentation as the animal matures reflects the anteroposterior and dorsoventral distribution pattern that has preceded melanogenesis (*61*).

Recently Goudie *et al.* (*62*) have used the regional variation in epidermal melanocytes in man to develop a more general hypothesis concerning the generation of positional information. They postulate that endothelial cells are arranged during development in monoclonal zones such that each clone is capable of imposing a developmental bias on the vascular tree and of conveying regional determinants that influence morphogenesis of the surrounding tissues. They argue that it is the vascular clones that provide the physical basis for explaining topographical systems by polar coordinates, and that their hypothesis "may account for the remarkable anatomical distribution of some neoplastic and non-neoplastic lesions." Their hypothesis, based on clinical observations, once again suggests a continuum between the development of the vascular system and the subsequent regional differences in normal and abnormal growth and development.

Most informative, in our attempt to carry the concept of developmental gradients to adult systems, has been the observation that mice given a lethal dose of x-irradiation and protected from the lethal effects by the administration of syngeneic bone marrow cells go through a change in hair pigmentation with increasing time after irradiation that serves as an indicator of an underlying regional pattern (Fig. 4). We observed that loss of pigment, seen as the animal gradually loses its agouti color and turns more and more to gray-white, occurs first in the thoracic region and then appears more cranially and caudally so that the final remaining agouti coloration is in the dorsal lumbosacral region (*28*). The pattern of pigment loss accurately mimics the pattern of tumor growth reported previously (*6*). A similar progressive pigment loss has also been reported by Popp (*63*) in a mutant subline of C3H mice. Popp found that this subline, which shows various symptoms of premature aging, exhibits a sequential depigmentation that starts in the thoracic region and gradually moves backward in an anteroposterior fashion.

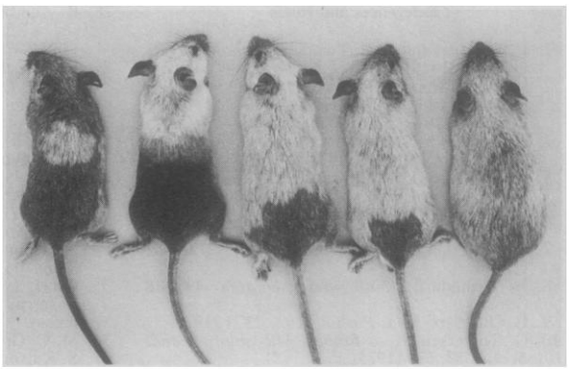

Fig. 4. Age-associated changes in pigmentation in bone-marrow protected, heavily irradiated mice over a 9-month period following irradiation. The earliest changes are seen in the mouse at the left (*28*).

Concluding Comments

In animal experimentation, protocols for the assessment of the efficacy of antineoplastic agents and the actions of hormones and growth inhibitors on tumors, as well as studies of cell and tissue interactions affecting tumor growth, usually involve tumor transplantation either intradermally, subcutaneously, or intraperitoneally. The results of such experiments can evidently be profoundly influenced by failure of the investigators to adhere strictly to defined methods of inoculation or to take into consideration the effects of topographical factors. Results of studies of carcinogenesis and cocarcinogens are also liable to be misleading if the location of inoculation or treatment sites is not strictly controlled.

Immunotherapy studies are particularly prone to erroneous interpretation because slight differences in the results brought about by regional differences in growth regulation may be further compounded by regional influences in immune responsiveness (*64, 65*). The efficacy of immunosuppressive agents and antigens, the generation of relative numbers of helper or suppressor cells, and other facets of immune responsiveness ranging from sensitization to antigen processing and effector cell generation may also be influenced both by topographical factors (*64*) and regional differences in immune responsiveness. It is now well known that a variety of vasoactive mediators, acting directly or indirectly to induce angiogenesis, are released as an accompaniment to both T-cell and macrophage activation (*66*).

The importance of topographical factors in determining the efficacy of cancer-controlling drugs is only one aspect of pharmacological research in general. This importance has been well documented by Vinegar's studies of drug toxicity (*67*). Development of any treatment regimen, ranging from administration of narcotics and anesthetics to inoculation of tracer substances must take regional factors into account.

These practical considerations are of immediate concern and deserve emphasis. However, the biological questions concerning the basic mechanisms that underlie the regional differences remain. There is a vast literature on other types of regional variation of biological phenomena: differences in blood flow to specific body organs and parts; morphological differences within the nervous system; location-specific variations in muscle tissue; polarity in virtually every organ; and a complexity of gradients that seems overwhelming. To search for an explanation of cephalocaudal or dorsoventral gradients in growth and differentiation is to walk in a scientific minefield. The challenge seems worth pursuing, however, because a fuller understanding of the processes underlying these regional differences might add substantively to our knowledge of the basic conditions under which growth takes place.

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Biological Control in Agroecosystems

Suzanne W. T. Batra

Crop yields in the United States are reduced by the effects of a variety of pests, including some 160 species of bacteria, 250 kinds of viruses, 8000 species of pathogenic fungi, 8000 species of insects, and 2000 species of weeds (*1*). Weeds are potentially the most damaging pests, followed by insects, plant pathogens, and nematodes (*2*). In spite of mechanized agriculture and advanced technology, losses of about 33 percent of potential production (*3*) or $20 billion annually (*4*) continue. Since 1945, agriculture has come to rely on synthetic chemicals (pesticides) for protection of crops. Usage of such chemicals has increased to over 544 million kilograms annually (*5*). Pesticides are valued for their uniform and rapid effectiveness, ease of application and shipment, and relatively long shelf-life. Unforeseen side effects, such as toxicity to nontarget organisms and induction of resistance in pests, have created a need to produce continually new pesticides, at a current cost of about $18 million each to develop, register, and market (*6*). For each pesticide marketed, more than 10,000 compounds may be screened (*7*) for effectiveness and safety.

Improvements in pesticides such as increased rate of degradation after application and more precise methods of application have made it possible to combine effectively the use of pesticides with other methods of pest control in integrated pest control programs. Public concern for environmental quality has led to increased emphasis on alternative pest management strategies, especially biological control. This is reflected in the fact that fundamental biology and nonchemical control projects accounted for almost 70 percent of the total funds recently budgeted by the U.S. Department of Agriculture (USDA) for pest control research (*8*). Diverse organisms, such as viruses, bacteria, fungi, rickettsiae, protozoa, nematodes, mites, insects, and vertebrates have all been used successfully as pest control agents in classical biological control, augmentative biological control, and conservative or natural biological control (Table 1). Each of these methods is based to a large extent on different ecological principles. In this

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