

CANCER-RELATED ASPECTS OF REGENERATION RESEARCH: A REVIEW

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Tissue regeneration is simply the replacement of lost cells of a tissue by those remaining. Epimorphic regeneration involves dedifferentiation of many tissues and their organization into a blastema which eventually differentiates into the missing part, usually an appendage.

A detailed comparison of the cell membrane changes occurring in epimorphic regeneration, tissue regeneration and cancer can contribute to greater understanding of the differences between normal and tumor cells. Further, there is evidence that epimorphic regeneration fields may in some instances suppress tumor induction and control existing tumors. This influence may be mediated by bioelectric fields, which are ubiquitous in nature and appear to control many cellular events. Disruption of these bioelectric fields suppresses epimorphic regeneration and may lead to cancer in mammals, while applied electric fields alter regenerative events and cause tumor regression.

INDEX WORDS: Regeneration, cancer

INTRODUCTION

The term *regeneration* has been used to describe replacement of appendages in vertebrates and invertebrates, repair of tissues such as muscle and skin, and restoration of visceral organs after their wounding or partial removal. While it is not always possible to categorize a given reparative event there are generally two types of regeneration, i.e., *tissue*, and *epimorphic*. Tissue regeneration is simply the replacement of lost cells and extracellular material by the remaining cells. Epimorphic regeneration, on the other hand, is the type found in regenerating appendages. It involves formation of a blastema, a mound of mesenchyme-like cells derived from dedifferentiation of numerous tissues adjacent to the amputation site. After a growth phase, the new parts arise by differentiation of the blastema, a more complicated process than tissue regeneration since many tissues must be integrated into a complex structure. Some organs such as liver seem to be restored by tissue regeneration while intestine appears to form a blastema. Tissue regeneration probably occurs to some extent in all animals but the epimorphic type is found only in certain species. The major part of this review is concerned with epimorphic regeneration in urodele

amphibians, but tissue regeneration in other vertebrates and epimorphic regeneration in invertebrates are also discussed.

The fate of dedifferentiated cells in limb and tail regeneration is highly controversial but some cells certainly transform to a type different from what they were originally (Steen, 1968). This also occurs in lens regeneration, a modified epimorphic process where dorsal iris epithelial cells lose their pigment, divide several times, and then transform to lens fibers. These facts coupled with recent observations that tissue regeneration involves antigenic changes in cell membranes (Hellstrom *et al.*, 1975; Manski and Whiteside, 1974) show that both types of regeneration provide opportunities to compare non-cancerous alterations in the differentiated state of adult cells to those occurring in neoplasia. Such comparisons should provide a clearer understanding of the tumor cell. Furthermore, the study of epimorphic regeneration reveals controls apparently basic to regulation of cell behavior and provides insight into possible mechanisms and management of neoplasia.

Although many of the ideas presented here are speculative, it is hoped that they will stimulate new lines of regeneration and cancer research.

RELATIONSHIP OF EPIMORPHIC REGENERATION FIELDS TO CANCER CONTROL

The relationship between regeneration and cancer has intrigued scientists for years. Waddington (1935) wondered whether a difference in cancer susceptibility exists between animals with a high and those with a low capacity for appendage regeneration. He further wondered about the behavior of tumor tissue transplanted to sites with varying regenerative potential. Needham (1936) was concerned with these same questions as well as the effects of carcinogens on regeneration. He suggested that "If the powerful growth stimulus of a carcinogenic substance acted within a powerful individuation field, the structures produced would show some sort of harmoniously regulated differentiation."

In 1948 Schlumberger and Lucké reviewed the existing literature on spontaneous tumors in amphibians, and noted that amphibians are no less susceptible to neoplasia than are other vertebrates. They listed seven reports of spontaneous tumors in urodeles, a form in which regeneration abilities are well developed.

A more recent review of spontaneous neoplasms in amphibia was presented by Balls (1962), who cited a total of only 58 accounts. The majority of new cases came from just 3 investigators leading him to believe that the apparent low incidence of amphibian tumors can be attributed to a lack of reporting. It is often difficult to turn one's attention to the occasional lump observed in experimental animals. He also suggested that since cancer is a disease of old age, few properly aged animals survive in nature and few investigators retain experimental animals long enough for tumors to develop. Balls reviewed fifteen reports of spontaneous tumors in urodeles and 43 in anurans. While these figures might suggest that urodeles are less susceptible to cancer than are anurans, Balls thinks once again that the difference is due to lack of investigation, since attempts to induce tumors chemically in urodeles have been more successful than in anurans (Leone, 1953, 1957, cited in Balls, 1962).

The above reports of urodele tumors reveal several instances of infiltrative tumors in regeneration fields. For example, Champy and Champy observed an invasive carcinoma of the skin of the flank and tail in *Triturus alpestrus* (cited in Schlumberger and Lucké, 1948) and Sheremetieva-Brunst and V. Brunst (1948) described spontaneous infiltrating melanomas in axolotl tails. These observations fail to support the concept of control of cancer by regeneration. However, the small sample (Table 1, Balls, 1962) does not provide a sound base from which to make conclusions about the possible effects of regeneration fields on neoplasia.

The relationship between cancer and regeneration is clearer when considering attempts to induce tumors chemically in urodeles. The review by Balls and Ruben (1964) contains only 2 reports of malignant tumors induced in non-epidermal limb tissues; both, spindle cell sarcomas. In the experiments of Breedis (1952) only two such tumors were generated from 500 injected animals. It is particularly instructive that these injections stimulated the formation of limbs rather than tumors, indicating a resistance of internal limb tissues to tumor formation. While the injected material caused degeneration of muscle and bone, this by itself, was not the stimulus for supernumerary limb formation since several carcinogens which caused the same degree of destruction differed in their supernumerary induction capacity. This suggests direct stimulation of blastema forming tissue by some car-

cinogens. The induced limbs gave no indication of harboring preneoplastic cells but regenerated in a normal manner, and when transplanted did not become established, in contrast to the ready transplantability of the sarcomas.

Strikingly similar results were obtained in a different system by Eguchi and Watanabe (1973). They implanted particles of the carcinogen N-methyl-N-nitro-N-nitrosoguanidine (NG) into lentectomized newt eyes. In 10 of 99 eyes, lenses regenerated not only from the dorsal half of the iris but also from the ventral half which normally is incapable of forming a lens. Simple lentectomy of 1000 eyes by these investigators never resulted in spontaneous supernumerary lens regeneration from ventral iris. In a further experiment, 7 of 60 pieces of ventral iris treated with an NG solution and then implanted into lentectomized eyes formed lenses or lens vesicles. In none of these experiments was there any evidence of carcinogenesis. NG apparently affected the ventral iris cells directly, releasing a portion of their genome. This effect persisted for at least a year since a second lentectomy of eyes with ventral lenses, led to lens regeneration from the same region of the ventral iris as before.

The experiments of Breedis, and Eguchi and Watanabe fulfill Needham's expectation that the stimulating effect of carcinogens in a regeneration field would result in "harmoniously regulated differentiation." There is further support for this view. Balls and Ruben (1964) noted that the most frequent response to carcinogens in amphibia is a varying hyperplastic response of the epidermis. They were reluctant to accept most of these as neoplasia but rather felt that they simply represented epithelial attempts to remove irritating substances. The following results of Seilern-Aspang and Kratochwil (1962, 1965), however, seem to involve true neoplasia and lend support to the concept of the controlling influence of regeneration fields. Subcutaneous injections of carcinogens were made into the mucous glands of the trunk, sacral region, and tail in *Triturus cristatus*. This resulted in numerous tumors of gland origin. In general, five stages of growth were observed (S-Aspang and Kratochwil 1962): 1. The germinal layer of the gland proliferated and filled the gland pocket, 2. The glands were perforated and the growths fused with each other and the overlying epithelium, 3. The tumor infiltrated the deeper tissues, 4. Some infiltrating tumors of the trunk penetrated and lined the peritoneal cavity, 5. Metastases

appeared under the skin in many parts of the body and in the viscera.

Tumors appeared in 16% of the animals injected in the trunk but in only 9% of those injected in the tail field (S-Aspang and Kratochwil, 1965). Trunk and sacral region tumors were all lethal, the majority infiltrating and giving rise to metastases. In contrast, few tail tumors showed this behavior, most healing spontaneously by differentiation of the cancer cells into a spherical structure containing all the layers of normal epithelium including a center of cornified cells. Just before their differentiation these tail tumors became partially surrounded by immature connective tissue cells. If these cells were destroyed with methylene blue injections, tail tumors did not differentiate but became lethal. Apparently this regeneration field controls epithelial tumors through interactions with the mesodermal tissues.

In further experiments (S-Aspang and Kratochwil, 1965) normal epithelium without its basement membrane was implanted into unamputated tails, limb blastemas and throat sac. In the tail, the implants largely degenerated. In the blastemas, the cells dedifferentiated and disappeared perhaps forming blastema cells as described by Rose and Rose (1965). In the throat sac, which is not a regeneration field, the epithelium began to infiltrate the muscle even more extensively than did the malignant tumors discussed earlier. This again shows control of invasive cells by regeneration fields.

The effects of regenerative processes on lymphoid tumors have been studied by several investigators with conflicting results. De Lustig and Matos (1972; Matos and De Lustig, 1973) induced tumorous nodules of lymphocyte-like cells in unamputated tails of *Bufo arenarum* tadpoles. These lymphomas ordinarily persisted until tail resorption at metamorphosis infiltrating and destroying muscle, neural tube and notochord. However, if the tails were amputated through the nodules, the tumor became encapsulated with a necrotic center, the periphery organized into tubular structures and finally the entire nodule disappeared. These results are different from those of Inoue and Singer (1963). They found a spontaneous lymphosarcoma in the liver of the newt, *Triturus*, and were able to propagate it by supernates of liver homogenates. Homogenate injections into limbs resulted in the development of local lymphosarcoma which metastasized to the viscera. Amputation of the tumor-containing limbs gave no evidence that the subsequent events would control the tumor, but rather, the tumor

usually prevented regeneration. When regeneration did occur, it was small and incomplete. In most of the limb segments removed at the time of amputation all the soft tissues had been replaced by tumor. This and the fact that many limbs remained ulcerated and were unable to form a wound epithelium, a necessary prerequisite for regeneration, indicate that the tumor was never exposed to a strong regeneration process.

Ruben and Balls (1964a) implanted lymphosarcoma into one limb of metamorphosed *Xenopus laevis* and amputated some of the contralateral limb. After an inflammatory reaction (presumably a homograft response against the implant), tumor cells appeared in both limbs, including the regenerate. In a further study (1964b) lymphosarcomas were induced in *Xenopus* limbs by implantation of methylcholanthrene crystals. Many tumors were found developing and growing in regenerates. The investigators concluded that lymphosarcomas do not come under control by the *Xenopus* regeneration field and suggested that only tumors derived from limb-competent cells can be controlled by limb fields and that non-competent ones such as lymphoid cells cannot be controlled. It should be noted however, that post-metamorphic *Xenopus* usually regenerate limbs rather poorly and represent a weak regeneration field. Regenerates on these forms are frequently composed of only a single spike with no digits and a number of fused wrist cartilages (Ruben and Balls, 1964a). This led De Lustig and Matos (1972) to suggest that their lymphoid tumors came under control and Ruben and Balls' did not because the larval *Bufo* tail is a stronger field than *Xenopus* limbs. This objection was considered and rejected by Ruben *et al.* (1966) when they observed simultaneous formation of supernumerary limb structures and lymphosarcoma in *Triturus* in response to frog kidney implants. Since *Triturus* limbs represent a good regeneration field they felt the objection was countered. However, their report does not indicate how complete the supernumerary limbs were. The pictures suggest that only tissue level "superregeneration" occurred. The implant may not have released the full complement of morphogenetic events and controls possible in this animal.

In addition to his experiments with lymphosarcoma, Ruben (1955, 1956) has amputated limbs of larval *Ambystoma* and adult *Triturus* which had previously been implanted with Lucké renal adenocarcinoma

of the frog. In no case did he observe dedifferentiation of tumor cells or their metaplasia into normal limb tissues. Perhaps the negative results were due to the genetic disparity between donor and host.

It was mentioned earlier that melanomas can develop spontaneously in a regeneration field. Axolotl tails amputated repeatedly through 8 such spontaneous tumors gave no evidence that melanoma cells transformed into other cell types (Sheremetieva, 1965), and melanoma cells often could be found in portions of the regenerate. Usually when a regenerate was melanized, the cells grew into it as regeneration was occurring. The ingrowth, however, was not of an invasive nature as is normally seen in non-regenerating tissue. Sheremetieva refers to it as a symbiotic participation by the melanoma.

To summarize the foregoing material, some tumors such as lymphosarcomas and melanomas can originate and grow in regeneration-competent appendages. Evidence exists that the growth of both of these tumors is modified to some extent by initiating regeneration of the appendages containing them. In the case of the lymphosarcoma, some situations (*Xenopus* limbs) show no control while others (*Bufo* tails) do. Melanomas appear to be less malignant when growing in new regenerates. Further evidence for control comes from the observations that iris and mesodermal tissues in regeneration fields are quite resistant to carcinogens and potentially lethal epithelial tumors spontaneously differentiate in the tail field.

It is most interesting that the usual response of mesodermal limb tissues to carcinogen injection or implantation is supernumerary limb formation. This was observed by Breedis (1952) and by Ruben and Balls (1964b). In the work reported by the latter investigators, methylcholanthrene induced lymphosarcomas as well as regenerates, which were interpreted as accessory limbs, suggesting that resistance to carcinogens is not a characteristic shared by all cells in these forms but is limited to normal participants in the regeneration field. Cells such as lymphocytes which are not normally part of the field, may be more difficult to control. The two induced sarcomas of Breedis are rare examples of limb mesodermal tumors. His further experiments show that on those few occasions when carcinogens alter limb cells so much that they form a tumor, regeneration processes can no longer control them (Breedis, 1954).

While much remains to be learned regarding the mechanisms which control blastema and potential cancer cells in these systems it is becoming clear that bioelectric fields play an important role.

BIOELECTRIC FIELDS IN REGENERATION AND CANCER

Bioelectric fields occur when an electrical potential difference exists between adjacent regions of an organism. Such fields are very common and are probably found in most normal living tissues. For example, they have been found in marine hydroids (Barth, 1934) plants (Lund and Rosene, 1947), and a variety of vertebrate tissues including those of humans (Burr, 1950; Athenstaedt, 1970). Further, cancerous regions of organs are generally negative to normal regions of these same organs, and there seems to be a correlation between degree of negativity and degree of malignancy (Schauble and Habal, 1970). There is good evidence showing that electric fields can control the behavior of cells. Marsh and Beams (1952) embedded pieces of planaria without heads or tails in agar and exposed them to an electric field so that the original head end was oriented either to the negative or positive pole. When the original head end was aligned with the negative pole, the original polarity was retained at all current densities, but with the original head end toward the positive pole, increasing the current gave increasing evidence of head formation in the tail. At lower currents, the tail end showed only temporary head behavior, while increasingly higher ones caused temporary head structures and behavior, permanent bipolars, and finally, at the highest currents, permanent axis reversal.

Measurement of surface potentials indicates that in some species of marine hydroids the regenerating distal end is negative to the middle of the stem and in some it is positive (Barth, 1934). Despite this difference in polarity, they share the common feature of inhibition of distal regeneration when sufficiently strong, externally applied currents are arranged so that the distal end faces a charge opposite to the one it normally has during regeneration (Barth, 1934). From the work of Rose it appears that bioelectric fields in the hydroid *Tubularia* regulate regeneration by providing a field in which positively charged informational molecules move along the animals' axis. The control seems to be one of inhibition such that as distal regions develop, they produce inhibitory substances which prevent more proximal regions from developing distal structures (Rose, 1957, 1963, 1966, 1970a,

1970b). For example, a regenerating distal region of one *Tubularian*, when grafted onto the distal region of a younger regenerate, suppresses formation of host distal structures if the graft is made with normal proximal-distal polarity but not if it is reversed (Rose, 1957). Apparently the inhibitory molecules normally move from distal to proximal but when the graft polarity is reversed, they cannot move into the host. However, if the host and normal-polarity graft are placed in an electric field with the grafted end facing the negative pole, the graft then has no effect, the negative pole apparently preventing positively charged inhibitory molecules from moving into the host (Rose, 1963).

It is well known primarily from the work of Singer and his associates that limb regeneration will not occur in the absence of nerves. Singer believes that nerves produce their effect through a trophic substance (Singer, 1974). Rose and Rose however, have proposed that nerves alter the limb bioelectric field thus allowing the proper polarized passage of informational molecules from non-nerve tissues (Rose, 1964; Rose and Rose, 1974b). In studies by Becker (1961), the limb polarity was mapped in regenerators (salamanders) and non-regenerators (frogs). After amputation, the salamander limb, which is originally negative to the area over the brain, becomes positive for the first few days, then becomes more negative than before amputation, maintaining this negativity until regeneration is complete. On the contrary, the amputated non-regenerating limb of the frog also becomes positive in the first few days but remains positive for many days as healing occurs. It becomes negative only after it heals and even then for a time is not as negative as the normal limb. That these measured fields are important to regeneration was shown by applying small external currents to regenerating larval salamander limbs for 5-10 minutes each day (Becker, 1961). Currents of the same polarity as that occurring naturally, accelerated the accompanying stage of regeneration.

Further evidence that electrical polarity is important in regenerative events was obtained when limb regeneration occurred in adult frogs whose limbs were amputated and implanted with the negative lead from a battery-powered electrical circuit placed under the skin of the back (Smith, 1974). Ordinarily frogs lose the ability to regenerate their limbs at metamorphosis, but in these experiments, the best case regenerated a complete functional hand. Similar results have been ob-

tained in rat limbs by Becker (1972a) using galvanic current generated by silver and platinum wire implants. The response was less than the formation of a regenerate with recognizable digits but was clearly an attempt at regeneration surpassing that of the controls. The best showed considerable regeneration of the amputated humerus even forming ossification centers in the regenerated epiphysis.

Becker and Murray (1967) have evidence that the bioelectric field alone may stimulate dedifferentiation. Amphibian red cells (which are nucleated) were exposed to a non-uniform electric field and subsequently began to regress through their original developmental sequence. The investigators believe that the trigger involves the cell membrane and have shown that once the process begins, it will continue even in the absence of any current. They further believe that these events normally occur in amphibian fracture healing with erythrocytes transforming to fibroblasts. These same techniques applied to human small lymphocytes cause them to revert to a more primitive blast element (Becker, '72b). The stimulus for the described behavior of amphibian red cells at fracture sites is thought to be the local electrical field produced by the fracture (Becker and Murray, '67). This thought is consistent with the generally accepted belief that stress to bone causes electrical fields which in turn lead to various cellular events, the usual description being that electronegativity occurs on the concave surface in association with osteoblasts and bone accretion, while positivity occurs on the convex surface and is associated with osteoclasts and bone resorption (Bassett, '72). Even tumors can be controlled by imposed electrical fields. Such a technique was used on mouse sarcoma 180 and 60% of the treated mice showed complete regression of the tumor while the controls all died (Humphrey and Seal, 1959).

From the foregoing discussion, it seems possible that if the regulation of normal cellular behavior is by way of bioelectric fields, then neoplasia may in some cases be triggered by disruptions in such fields. There is evidence to suggest that abnormal local polarities stimulate neoplasia. Implantation of plastic films into the subcutaneous tissues of rats was found to stimulate sarcoma formation significantly more often if the films carried a net surface charge, with cationic more carcinogenic than anionic films (Carter *et al.*, 1971). Surface charge

was also implicated in the induction of anaplastic fibrosarcomas of mice implanted subcutaneously with two types of millipore filters (Andrews, 1972). More tumors occurred around filters with a negative charge than those with a positive one.

Disruption of bioelectric fields may also explain some cases of x-ray induced carcinogenesis.

Bioelectric Fields and X-ray

The paradoxical feature of x-rays in both causation and cure of cancer is well known. The most commonly held theory of x-ray induced carcinogenesis is that it is mutagenic. The theory that neoplasia involves at least one initiator mutation and possibly several cumulative ones has recently been reviewed by Knudson (1973). He suggests that the most likely regions of the genome that would lead to the abnormal characteristics seen in cancer cells would be those controlling the cell surface. Studies of the effects of x-rays on regeneration suppression suggest that there are other than mutagenic effects at work here. Certainly the failure of a large number of cells to initiate regenerative processes after x-ray treatment cannot be due to random mutation. Nor can it be due to destruction of the mitotic capability of the irradiated cells, since epidermal cells continue to divide in an apparently normal manner (Rose and Rose, 1965) and there is no reason to think that epidermis would be less radiosensitive than the internal tissues. In fact because dividing cells are more sensitive to radiation than non-dividing ones, the epidermis should be more sensitive than the mesodermal limb tissues, which rarely divide. Furthermore, there is evidence that under certain circumstances, even internal limb cells can divide after irradiation (Desha, 1974). It appears that x-rays may act by disrupting the bioelectric field. Rose and Rose (1974a) found that doses of x-rays that suppress regeneration in innervated limbs do not stop it in aneurogenic ones. Aneurogenic limbs have never had nerves and consequently will regenerate without them. The main point of this experiment is that x-rays appear to be interfering with some neurally-mediated aspect of the regeneration process. Polarity measurements of normal and x-rayed limbs suggest that the disruption is related to the bioelectric field (Rose and Rose, 1974b). If a series of 4 points is measured along the proximo-

distal axis of a normally regenerating limb, the distal-most point is negative to the second, the second is negative to the third and the third is positive to the fourth (— +). However, in x-rayed limbs the pattern is — — —.

X-irradiation also appears to damage morphogenetic mechanisms (Oberpriller, 1968 and Carlson, 1974). Oberpriller first confirmed earlier work showing that two regenerating limbs fused parallel to each other regenerate normally; but when the distal ends meet at an angle, there is a reduction in digit number where the two fields overlap. She then found that when one of the fused limbs is irradiated, it no longer influences regeneration of the other. This work shows that x-rays can block the ability of the stump to control blastema cells.

In Carlson's experiments, skin cuffs were exchanged between left and right limbs so that the anterior-posterior skin axes were reversed. Amputation of these limbs resulted in multiple regenerates unless the skin was irradiated, then only single regenerates formed. Mismatching the axes of skin and deeper tissues apparently provides the regenerate with two information centers which cannot be integrated. Irradiation of the skin blocks its ability to act as an organizing center.

We have already alluded to the induction of neoplasms by abnormal local polarities in the form of charged plastic films or millipore filters. The experiments just described suggest that x-rays may in some cases, cause cancer by disrupting normal bioelectric polarities and thus the means by which cell behavior is normally controlled. The fact that x-rayed newt limbs have not been reported more susceptible than normal to neoplastic processes does not necessarily negate this possibility. The lack of evidence may stem from several causes: first, x-rayed animals may not have been retained long enough to detect spontaneous tumors; second, we know of no attempts to test this hypothesis directly using carcinogens on x-rayed limbs; and finally, there is evidence to suggest that in addition to the ability of regeneration fields to control cancer there seems to be a second systemic mechanism operating in Urodeles that makes them relatively refractory to neoplasia (Ingram, 1971).

An important determinant of cell behavior involves the cell surface. It is becoming evident that significant and possibly similar changes occur in the plasma membranes of cancer cells and cells involved in epimorphic and tissue level regeneration.

Relationship of Tumor Cell Membranes to Those of Regeneration Cells

Immunization of guinea pigs with unfertilized mouse eggs generates an antiserum cytotoxic to the eggs used for immunization as well as to SV 40 transformed embryonic mouse cells but not the normal counterpart of the transformed cells. Immunofluorescence studies show that the antibodies are directed against a cell surface component. These experiments indicate that malignancy is sometimes associated with the reappearance of membrane antigens normally present in an earlier stage of development (Baranska *et al.*, 1970). In other experiments along these lines (Coggin *et al.*, 1970) hamsters were immunized with IP injections of irradiated living cells from hamster fetuses of various ages. Different cell types in diffusion chambers were then implanted IP into the immunized animals and subsequent growth of the implants assessed. In animals immunized with the earlier stages of development, growth of SV 40-transformed hamster cells were suppressed as were earlier embryo cells. Normal adult kidney cells were unaffected and later embryo cells affected very little. These experiments show that fetal cells possess membrane antigens that are normally lost as development proceeds. It further shows that these antigens reappear in SV 40-transformed cells. It is not clear whether the induced antigens in the above studies represent newly synthesized material or are simply the exposure of substances that were on the cell surface in early development but were later hidden.

In order to understand fully the significance of embryonic antigen reappearance in tumor cells it is necessary to know if similar changes can occur in normal cells as they respond to various stimuli. If such antigens could be demonstrated on blastema cells, it would indicate that such behavior is not tumor specific. It is clear that during limb and tail regeneration, new proteins and/or new forms of old proteins appear (Schmidt, 1966, 1968; Donaldson *et al.*, 1974; Dearlove and Stocum, 1974). Furthermore, antigens undetectable in normal rat skeletal muscle have been found during its regeneration (Khudaidatov, 1965, 1966). At present the identity and cellular distribution of these proteins is unknown. Some of them may be recurring embryonic membrane proteins. One of us has recently begun to study the problem of embryonic antigens in regeneration using the intestine of adult frogs

(Donaldson *et al.*, 1973). Antisera were made against early frog embryos and after absorption with adult organs and tissues, were reacted against extracts of regenerating intestine. The results revealed that eggs and embryos possess some antigens not present in any adult organs and at least one also present in adult skin. When the absorbed antisera containing these antibodies were reacted against extracts of regenerating intestine no reaction occurred. It is interesting to compare these results to those of Stonehill and Benditch (1970), who found an antigen present only in mouse embryos, 72 different tumors and adult skin. We found a similar antigen in frogs but could not demonstrate its presence in intestinal regenerates. Since it is very difficult to prove serologically that antigens are absent, it cannot be concluded that blastema cells are without embryonic antigens. Further studies are needed involving more sensitive immunological techniques, different embryonic stages for antibody production, different regeneration systems, and different extraction media to solubilize proteins not available with saline methods.

A statement in a paper by Ruben and Balls (1964a) suggests that surface antigenic changes do occur in epimorphic regeneration. They state that strong systemic reactions in *Triturus* against large implants of *Rana pipiens* tissue will prevent regeneration for up to 21 days, and that growth rather than differentiation is most affected. Superficially this seems similar to the cancer immunity seen in animals whose immune system has been non-specifically stimulated by BCG (Mathe *et al.*, 1969; Parr, 1972) or whose macrophage system has been similarly armed by various parasitic infections (Hibbs *et al.*, 1972; Hibbs, 1973), tumor cell rejection apparently occurring because of their abnormal antigenic surfaces. The details of Ruben and Balls' work are unpublished but suggest that blastema cells have surfaces unlike differentiated cells.

Some membrane changes during tissue regeneration involve the exposure of normally hidden membrane components (Manski and Whiteside, 1974). Normal and regenerating corneal epithelium were exposed to a fluorescein-labeled serum raised against normal cornea. The anti-serum stained the regenerating cells but not the normal ones. Since the antiserum was generated from extracts of normal cornea but did not react with intact normal cells, it was concluded that the antigen is present in normal cells but not at the surface. This work suggests

that some "new" antigens in cancer cells may be expressed by similar mechanisms and might explain the reappearance of embryonic antigens in tumors.

Recent work by Hellstrom *et al.* (1975) provides additional evidence that tissue regeneration changes the antigenic characteristics of the cell surface. Their work further shows that the changes may be similar to those in neoplasia. Lymph node cells from mice undergoing liver regeneration became consistently cytotoxic to cultured sarcoma cells but not their normal counterparts. The authors suggested that during liver regeneration, undifferentiated cells appeared whose antigens immunized the host against the cancer cells. They also found that a serum antibody was produced that could block this cell-mediated cytotoxicity. Blocking antibody might be a means of protecting liver cells from lymphocytes during regeneration, but it may also protect tumor cells. It would seem that a more useful method of dealing with regeneration cell surfaces would be by morphogenetic controls such as those operative in epimorphic regeneration.

A different way of studying the normal and abnormal cell surface is by the use of plant lectins, such as concanavalin A (Con A), a multivalent jack bean protein with affinity for glucose or mannose-like sites (Sharon & Lis, 1972). Tissue culture cells transformed by viruses or carcinogens show loss of contact inhibition, that is, they continue to grow even when a confluent monolayer is formed (Aaronson and Todaro, 1968). This abnormal behavior of transformed cells is accompanied by an increase in their agglutinability by Con A (Inbar and Sachs, 1969). A direct relationship between Con A agglutinability and malignancy is suggested by the observation that variants of transformed cells which no longer show in-vitro transformation properties also show loss of agglutinability (Inbar *et al.*, 1969), and by studies correlating agglutinability with tumorigenicity (Inbar *et al.*, 1972). These observations and the fact that light proteolysis of normal cells increases their agglutinability by Con A and causes them to transiently escape contact inhibition (Burger, 1970) suggest that some cancer cells are simply normal cells that have permanently lost a surface protein that normally promotes contact inhibition and prevents agglutination by Con A. Indeed, a contact inhibitory protein has recently been isolated (Lipkin and Knecht, 1974). The additional fact that Con A induces receptor clustering on transformed

and trypsinized cells but not normal ones (Nicolson, 1973) suggests that this same protein (or others) may stabilize membrane fluidity. When these proteins are lost either by transformation or proteolysis, rearrangement of membrane materials may occur resulting in abnormal behavior.

Loss of membrane material may be a prerequisite for any significant change in cell behavior since it also accompanies dedifferentiation of epithelial cells during lens regeneration (Zalik and Scott, 1972, 1973). The electrophoretic mobility (EPM) of trypsin-dissociated cells from various stages of lens regeneration was determined, and it was found that during dedifferentiation (10-day regenerates), EPM values were lower than normal iris cells or those from earlier stages. As regeneration progressed, the values gradually increased back to normal. Treatment of various trypsin-dissociated stages with a number of other enzymes revealed that the normal cells and those from the first few days of regeneration could have their EPM reduced but later stages prior to lens fiber differentiation were unaffected. These results seem to indicate that as lens regeneration occurs, certain surface components responsible for normal EPM are lost from the cell. Thus, in the early stages of regeneration when this component is still present, further enzyme treatment removed it, changing the EPM. Later, when this component disappeared, the enzymes could have no further effect. It does not appear that the results were due to differential release of surface material of some stages by the trypsin used for dissociation since mechanically dissociated cells behaved similarly (Zalik and Scott, 1973). Further experiments indicated that there was a sequential disappearance of at least two different surface components. One (or more) components, sensitive to RNase, hyaluronidase, and chondroitinase were absent by 5 days while neuraminidase-sensitive materials were not absent until after 10 days.

It is tempting to think that the loss of surface material during lens regeneration may have something to do with initiation of mitosis and dedifferentiation. Indeed, light proteolysis will initiate DNA synthesis in dissociated cells of sea urchin embryos (Vitorelli *et al.*, 1973). Loss of specific proteins (although not necessarily from membranes) has also been observed in wound healing and limb and tail regeneration (Schmidt, 1966, 1968; Donaldson *et al.*, 1974; Dearlove and Stocum, 1974). It would be interesting to know what the relationships are be-

tween the membrane material lost during dedifferentiation and during neoplastic transformation. Burger and Noonan believe that the cell cover is non-specific, the important part being the exposed layer (Burger and Noonan, 1970).

As yet there have been no studies of plant lectin binding sites during regeneration; undoubtedly these will be forthcoming. They are needed to complement observations showing similarities of embryonic cell membranes and those of transformed cell lines. Embryonic chick retinal cells readily agglutinate with Con A, but adult retinal cells will not (Kleinschuster and Moscona, 1972). Similarly, Con A easily agglutinates intestinal epithelial cells from the human fetus but not the adult (Weiser, 1972). Does this mean that post-natal tumors arise because organisms have lost a mechanism possessed by embryos for controlling cells with embryonic characteristics? It would be most interesting to know if during epimorphic regeneration, dedifferentiated cells show increased Con A agglutinability. If they do, it might imply that adult salamanders retain the ability to control cells with embryonic surfaces, a theory consistent with the ability of regeneration fields to control epidermal tumors and prevent chemically induced neoplasia. It should be noted however, that the proposed similarity between embryonic and transformed cells has yet to stand the test of whether embryonic agglutinability is accompanied by induction of receptor clusters. Since agglutination is a complex phenomenon influenced by many factors, it is necessary to establish that membrane fluidity is similar in embryonic and transformed cells. These same reservations would apply to experiments simply showing increased Con A agglutinability following dedifferentiation during regeneration.

In summary, it appears that neoplasia as well as tissue and epimorphic regeneration are accompanied by important and perhaps similar membrane changes. Transformed cells may show "new" surface antigens some of which were present during embryonic development. Suppression of epimorphic regeneration during systemic reactions against foreign tissue implants suggests that the blastema cell surface is also antigenically altered. The fact that liver regeneration in mice immunizes them against cancer cells indicates that here too, new membrane antigens appear during regeneration and additionally implies a similarity between surfaces of regenerating liver cells and cancer cells. The surfacing of "hidden" membrane antigens during normal corneal

epithelial cell regeneration suggests that some new antigens including those on cancer cells may actually represent a rearrangement of the membrane without synthesis of new material. Experiments with plant lectins have shown that mobility of membrane components increases in cancer cells, an alteration which would facilitate rearrangement of membrane components. This membrane fluidity may well be promoted by loss of proteins such as the one isolated by Lipkin and Knecht. Loss of membrane material during lens regeneration suggests that this is necessary for altered cell behavior.

Since membrane changes in cancer cells are clearly important in their abnormal behavior, it is essential that the similarities and differences of cancer cell and regeneration cell membranes be delineated. The key to understanding the abnormal cell surface may lie in understanding that of the regeneration cell. It is also important to study the response of the organism to the regeneration cell surface. The blocking antibodies in mammals that protect regeneration cells from destruction by lymphocytes may also protect the neoplastic cell with its similar surface. In contrast, the immune system of amphibians may not normally be sensitive enough to the altered surfaces of blastema cells to require their protection by blocking antibody. In these organisms some potential cancer cells within regeneration fields may have surfaces similar to blastema cells and therefore come under the same morphogenetic influences.

REFERENCES CITED

- AARONSON, S. A., & TODARO, G. J. 1968. Basis for the acquisition of malignant potential by mouse cells cultivated in vitro. *Science* **162**, 1024-1026.
- ANDREWS, E. J. 1972. Possible importance of detergent in millipore filter carcinogenesis. *J. Natl. Cancer Inst.* **48**, 1251-1254.
- ATHENSTAEDT, H. 1970. Permanent longitudinal electric polarization and pyro-electric behavior of collagenous structures and nervous tissue in man and other vertebrates. *Nature* **228**, 830-834.
- BALLS, M. 1962. Spontaneous neoplasms in amphibia: A review and description of 6 new cases. *Cancer Res.* **22**, 1142-1154.
- BALLS, M., & RUBEN, L. N. 1964. A review of the chemical induction of neoplasms in amphibia. *Experientia* **20**, 241-247.
- BARANSKA, W., KOLDOVSKY, P., & KOPROWSKI, H. 1970. Antigenic study of unfertilized mouse eggs: cross reactivity with SV40-induced antigens. *Proc. Natl. Acad. Sci.* **67**, 193-199.
- BARTH, L. G. 1934. The direction and magnitude of potential differences in certain hydroids. *Physiol. Zool.* **7**, 365-399.
- BASSETT, C. A. L. 1972. A biophysical approach to craniofacial morphogenesis. *Acta Morphol. Neerl-Scand.* **10**, 71-86.

- BECKER, R. O. 1961. The bioelectric factors in amphibian limb regeneration. *J. Bone and Joint Surg.* **43A**, 643-656.
- . 1972a. Stimulation of partial limb regeneration in rats. *Nature* **235**, 109-111.
- . 1972b. Augmentation of regenerative healing in man. A possible alternative to prosthetic implantation. *Clin. Orth. Rel. Res.* **83**, 255-262.
- BECKER, R. O., & MURRAY, D. G. 1967. A method for producing cellular dedifferentiation by means of very small electrical currents. *Trans. N.Y. Acad. Sci.* **29**, 606-615.
- BREEDIS, C. 1952. Induction of accessory limbs and of sarcoma in the newt (*Triturus viridescens*) with carcinogenic substances. *Cancer Res.* **12**, 861-866.
- . 1954. Effect of temperature on a neoplasm-regenerate complex in the newt (*Triturus viridescens*). *Fed. Proc.* **13**, Abstr. 1390, p. 425.
- BURGER, M. M. 1970. Proteolytic enzymes initiating cell division and escape from contact inhibition of growth. *Nature* **227**, 170-171.
- BURGER, M. M., & NOONAN, K. D. 1970. Restoration of normal growth by covering of agglutinin sites on tumor cell surface. *Nature* **228**, 512-515.
- BURR, H. S. 1950. Bioelectricity: potential gradients. In: *Medical Physics* vol. 2, p. 90-94, Otto Glasser ed., Chicago, Yearbook Publ. Co.
- CARLSON, B. M. 1974. Morphogenetic interactions between rotated skin cuffs and underlying stump tissues in regenerating axolotl forelimbs. *Dev. Biol.* **39**, 263-285.
- CARTER, R. L., ROE, F. J., & PETO, R. 1971. Tumor induction by plastic films: Attempt to correlate carcinogenic activity with certain physicochemical properties of the implant. *J. Natl. Cancer Inst.* **46**, 1277-1289.
- COGGIN, J. H., AMBROSE, K. R., & ANDERSON, N. G. 1970. Fetal antigen capable of inducing transplantation immunity against SV40 hamster tumor cells. *J. Immun.* **105**, 524-526.
- DEARLOVE, G. E., & STOCUM, D. L. 1974. Denervation-induced changes in soluble protein content during forelimb regeneration in the adult newt, *Notophthalmus viridescens*. *J. Exp. Zool.* **190**, 317-328.
- DE LUSTIG, E. S., & MATOS, E. L. 1972. Regeneration and cancerogenesis: Carcinogenesis in regenerating tadpoles tails of *Bufo arenarum*. In: First conference on cell differentiation. Munksgaard, Copenhagen, pp. 124-130.
- DESHA, D. L. 1974. Irradiated cells and blastema formation in the adult newt, *Notophthalmus viridescens*. *J. Embryol. Exp. Morph.* **32**, 405-416.
- DONALDSON, DONALD J., MINCHEY, J. W., & ADCOCK, K. 1973. A search for embryonic antigens in regenerating intestine of the adult leopard frog, *Rana pipiens*. *Oncology* **28**, 523-535.
- DONALDSON, D. J., MASON, J. M., & JENNINGS, B. R. 1974. Protein patterns during regeneration and wound healing in the adult newt: A comparative study using gel electrophoresis. *Oncology* **30**, 334-346.
- EGUCHI, G., & WATANABE, K. 1973. Elicitation of lens formation from the 'ventral iris' epithelium of the newt by a carcinogen, N-methyl-N-nitro-N-nitrosoguanidine. *J. Embryol. Exp. Morph.* **30**, 63-71.
- HELLSTROM, I., HELLMSTRÖM, K. E., & NISHIOKA, M. 1975. Reactivity to tumor-associated antigens detected in mice undergoing liver regeneration. *Nature* **253**, 744-746.
- HIBBS, J. B., JR. 1973. Macrophage non-immunologic recognition: Target cell factors related to contact inhibition. *Science* **180**, 868-870.
- HIBBS, J. B., JR., LAMBERT, JR., L. H., & REMINGTON, J. S. 1972. Control of carcinogenesis: A possible role for the activated macrophage. *Science* **177**, 998-1000.
- HUMPHREY, C. E., & SEAL, E. H. 1959. Biophysical approach toward tumor regression in mice. *Science* **130**, 388-390.

- INBAR, M., & SACHS, L. 1969. Interaction of the carbohydrate-binding protein concanavalin A with normal and transformed cells. *Proc. Natl. Acad. Sci.* **63**, 1418-1425.
- INBAR, M., RABINOWITZ, Z., & SACHS, L. 1969. The formation of variants with a reversion of properties of transformed cells. III. Reversion of the structure of the cell surface membrane. *Int. J. Cancer* **4**, 690-696.
- INBAR, M., BEN-BASSAT, H., & SACHS, L. 1972. Membrane changes associated with malignancy. *Nature New Biol.* **236**, 3-4.
- INGRAM, A. J. 1971. The reactions to carcinogens in the axolotl (*Ambystoma mexicanum*) in relation to the regeneration field control hypothesis. *J. Embryol. Exp. Morph.* **26**, 425-441.
- INOUE, S., & SINGER, M. 1963. Transmissibility and some histopathology of a spontaneously originated visceral tumor in the newt *Triturus pyrrhogaster*. *Cancer Res.* **23**, 1679-1684.
- KHUDAIDATOV, I. S. 1965. Detection of stage-specific antigens in regenerating muscle tissue. *Byull. Eksp. Biol. Med.* (in Russian with English summary) **59**, 75-79.
- . 1966. Antigenic properties of regenerating muscle tissue. *Byull. Eksp. Biol. Med.* (in Russian with English summary) **62**, 103-105.
- KLEINSCHUSTER, S. J. & MOSCONA, A. A. 1972. Interactions of embryonic and fetal neural retina cells with carbohydrate-binding phytoagglutinins: cell surface changes with differentiation. *Exp. Cell Res.* **70**, 397-410.
- KNUDSON, A. G. 1973. Mutation and Human Cancer in: *Advances in Cancer Res.* **17**, 317-352.
- LIPKIN, G., & KNECHT, M. E. 1974. A diffusible factor restoring contact inhibition of growth to malignant melanocytes. *Proc. Natl. Acad. Sci.* **71**, 849-853.
- LUND, E. J., & ROSENE, H. F. 1947. Bioelectric fields and growth—with a bibliography of continuous bioelectric fields in animals and plants. U. of Texas Press, Austin.
- MANSKI, W., & WHITESIDE, T. L. 1974. Cell surface receptors of normal, regenerating and cultured corneal epithelial and endothelial cells. *Inves. Ophth.* **13**, 935-944.
- MARSH, G., & BEAMS, H. W. 1952. Electrical control of morphogenesis in regenerating *Dugesia tigrina*. I. Relation of axial polarity to field strength. *J. Cell and Comp. Physiol.* **39**, 191-213.
- MATHÉ, G., POUILLART, P., & LAPEYLAQUE, F. 1969. Active immunotherapy of L 1210 leukemia applied after the graft of tumor cells. *Br. J. Cancer* **23**, 814-824.
- MATOS, E. L., & DE LUSTIG, E. S. 1973. Teratogenic effects of carcinogen implantation in a regenerative field in *Bufo arenarum* tadpoles. *Teratology* **8**, 167-174.
- NEEDHAM, J. 1936. New advances in the chemistry and biology of organized growth. *Proc. R. Soc. of Medicine* **29**, 1577-1626.
- NICOLSON, G. L. 1973. Temperature dependent mobility of Concanavalin A sites on tumour cell surfaces. *Nature New Biol.* **243**, 218-220.
- OBERPRILLER, J. 1968. The action of x-irradiation on the regeneration field of the forelimb of the adult newt, *Diemictylus viridescens*. *J. Exp. Zool.* **168**, 403-422.
- PARR, I. 1972. Response of syngeneic murine lymphomata to immunotherapy in relation to the antigenicity of the tumor. *Br. J. Cancer* **26**, 174-182.
- ROSE, F. C., & ROSE, S. M. 1965. The role of normal epidermis in recovery of regenerative ability in x-rayed limbs of *Triturus*. *Growth* **29**, 361-393.
- ROSE, F. C., & ROSE, S. M. 1974a. Regeneration of aneurogenic limbs of salamander larvae after x-irradiation. *Growth* **38**, 97-108.
- ROSE, S. M., & ROSE, F. C. 1974b. Electrical studies on normally regenerating, on x-rayed and on denervated limb stumps of *Triturus*. *Growth* **38**, 363-380.
- ROSE, S. M. 1957. Polarized inhibitory effects during regeneration in *Tubularia*. *J. Morph.* **100**, 187-206.

- . 1963. Polarized control of regional structure in *Tubularia*. *Devel. Biol.* **7**, 488-501.
- . 1964. Regeneration. In: *Physiology of the amphibia*. Chapt. 10. J. Moore, Ed., Academic Press, New York.
- . 1966. Polarized inhibitory control of regional differentiation during regeneration in *Tubularia*: II. Separation of active materials by electrophoresis. *Growth* **30**, 429-447.
- . 1970a. Restoration of regenerative ability in ligated stems of *Tubularia* in an electric field. *Biol. Bull.* **138**, 344-353.
- . 1970b. Differentiation during regeneration caused by migration of repressors in bioelectric fields. *Amer. Zool.* **10**, 91-99.
- RUBEN, L. N. 1955. The effects of implanting anuran cancer into non-regenerating and regenerating larval urodele limbs. *J. Exp. Zool.* **128**, 29-51.
- . 1956. The effects of implanting anuran cancer into regenerating adult urodele limbs. I. Simple regenerating systems. *J. Morph.* **98**, 389-403.
- RUBEN, L. N., & BALLS, M. 1964a. The implantation of lymphosarcoma of *Xenopus laevis* into regenerating and non-regenerating forelimbs of that species. *J. Morph.* **115**, 225-238.
- . 1964b. The implantation of methylcholanthrene crystals into regenerating and non-regenerating forelimbs of *Xenopus laevis*. *J. Morph.* **115**, 239-254.
- RUBEN, L. N., BALLS, M., & STEVENS, J. 1966. Cancer and super-regeneration in *Triturus viridescens* limbs. *Experientia* **22**, 260-261.
- SCHAUBLE, M. K., & HABAL, M. B. 1970. Electropotentials of surgical specimens. *Arch. Path.* **90**, 411-415.
- SCHLUMBERGER, H. G., & LUCKÉ, B. 1948. Tumors of fishes amphibians and reptiles. *Cancer* **8**, 657-754.
- SCHMIDT, A. J. 1966. Electrophoretic separation of soluble proteins extracted from regenerating forelimbs of the adult newt, *Diemictylus viridescens*. *Anat. Rec.* **154**, 417-418.
- . 1968. Cellular biology of vertebrate regeneration and repair. U. of Chicago Press, Chicago, pp. 177-178.
- SEILERN ASPANG, F., & KRATOCHWIL, K. 1962. Induction and differentiation of an epithelial tumor in the newt (*Triturus cristatus*). *J. Embryol. Exp. Morph.* **10**, 337-356.
- . 1965. Relation between regeneration and tumor growth. In: *Regeneration in animals and related problems*. V. Kiortsis and H. A. L. Trampusch, eds. Amsterdam: No. Holland Publishing Co., pp. 452-473.
- SHARON, N., & LIS, H. 1972. Lectins: Cell-agglutinating and sugar-specific proteins. *Science* **177**, 949-959.
- SHEREMETIEVA, E. A. 1965. Spontaneous melanoma in regenerating tails of axolotls. *J. Exp. Zool.* **158**, 101-122.
- SHEREMETIEVA-BRUNST, E. A., & BRUNST, V. V. 1948. Origin and transplantation of a melanotic tumor in the axolotl. In: *The biology of melanomas*. *Spec. Pub. N.Y. Acad. Sci.* **4**, 269-287.
- SINGER, M. 1974. Neurotrophic control of limb regeneration in the newt. *Annals N.Y. Acad. Sci.* **228**, 308-322.
- SMITH, S. D. 1974. Effects of electrode placement on stimulation of adult frog limb regeneration. *Annals N.Y. Acad. Sci.* **238**, 500-507.
- STEEN, T. P. 1968. Stability of chondrocyte differentiation and contribution of muscle to cartilage during limb regeneration in the axolotl (*Sireodon mexicanum*). *J. Exp. Zool.* **167**, 49-78.

- STONEHILL, E. H., & BENDICH, A. 1970. Retrogenetic expression: the reappearance of embryonal antigens in cancer cells. *Nature* **228**, 370-371.
- VITORELLI, M. L., CANNIZZARO, G., & GUIDICE, G. 1973. Trypsin treatment of cells dissociated from sea urchin embryos elicits DNA synthesis. *Cell Diff.* **2**, 279-284.
- WADDINGTON, C. H. 1935. Cancer and the theory of organizers. *Nature Lond.* **135**, 606-608.
- WEISER, M. M. 1972. Concanavalin A agglutination of intestinal cells from the human fetus. *Science* **177**, 525-526.
- ZALIK, S. E., & SCOTT, V. 1972. Cell surface changes during dedifferentiation in the metaplastic transformation of iris into lens. *J. Cell Biol.* **55**, 134-147.
- . 1973. Sequential disappearance of cell surface components during dedifferentiation in lens regeneration. *Nature New Biol.* **244**, 212-214.