INTRODUCTION

The demands for energy-rich metabolites by particular organs during pre-natal growth and development are so great that the entire resources of the embryo are mobilized at specific times. There is an increased rate of activity of the major metabolic pathways during embryonic development on demand by particular sites. The product or result of this particular activity is growth of the organ or organs in question, and the requirements of the anlagen of teeth during the formation of the pre-enamel and pre-dentine matrices are no different. During such times any environmental insult resulting in a lack of available metabolites may produce congenital anomalies in those teeth upon which the embryo is at that time concentrating.

Tumors consume large amounts of energy for they have a higher aerobic and anaerobic glycolysis than most normal tissues. Warburg states that tumor cells utilize the energy of carbohydrate breakdown from a synthesis of cellular proteins to a larger extent than most normal tissues.

In transplanting spontaneous mammary tumors with normal tissues of different strains of mice, Browning observed there was for a time a stimulation of the normal tissue growth. As the alien tumor cells began to invade the growing embryonic homotransplants, focal concentrations of lymphocytes appeared which aided growth of the embryonic tissues by destroying the tumor cells. In studying the interaction between transplanted normal and malignant suspensions of cells, Schneyer did not obtain results similar to Browning. Oker-Blom and Alfthan had sporadic success with normal and neoplastic tissues dually transplanted to the chorio-allantoic membrane. It was observed by Malmgren's et al. that inter-strain tumor transplants failed to survive in tumors of a different genetic derivation, but when they

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were of the same genetic derivation, the tumor implants did survive. Grobstein and Younger\(^a\) concluded that tissue from two different species of animals may be grown simultaneously in flask cultures without apparent incompatibility.

There have been no reports on the intra-ocular transplantation of a human neoplasm with embryonic tissues (tooth germs) in experimental animals.

**METHODS**

The techniques of tumor\(^a\) and of tooth germ transplantation\(^a\) have been described in detail.

Several other transplantable tumors were employed in pilot studies with tooth germs, but these neoplasms outgrew and destroyed the tooth germ transfers. These tumors were Sarcoma 37, MT 65, and Sarcoma 180.

The human tumor used in these experiments was a glioblastoma multiforme and was obtained from a patient in the hospital operating room. For several years it has been carried serially in our laboratories in experimental animals. Its behavior upon transplantation to the eyes and/or brains of rabbits, rats, mice, and guinea pigs has been extensively studied.\(^9\) After a latent period of 20-30 days in the eyes or brains of the above-mentioned experimental animals, its growth is continuous and expansive. The tumor cells that survive transplantation are spongioblasts along with more primitive glial cells and giant cells. Before transplantation, necrotic tumor tissue and normal brain tissue were discarded and only fresh viable tumor in pieces of 1-1.5 mm. square were used in these studies.

Tooth germs were obtained from 25-35-day-old guinea pig embryos. The embryo or embryos of a single pregnant animal furnished the tooth germs for each series. On this basis, 27 different groups or series of varying numbers of young adult guinea pigs received transplants.

Each series was subdivided into three groups of animals. One group received tumor tissue alone, a second group received tooth germs alone, and the third group received tooth germs with tumor tissue. In this latter group, a total of 235 young adults of both sexes received transplants of tumor and tooth germs. A total of 112 animals received anterior chamber transplants of tumor tissue and 135 animals received transplants of tooth germs.

Tumor and tooth germs were allowed to grow together for varying periods from 35-350 days. Control animals, bearing only tooth germ transplants, were kept for periods varying from 35-125 days. Hosts with tumor tissue only were not sacrificed until the corneas were about to rupture. This usually occurred from 75-90 days following transplantation.

Moribund hosts were sacrificed at once and transplanted tissues, when present, recovered. Animals that became sick, developed eye infections, or failed to show evidences of the growth of transplants were discarded. These are listed in Table A but are deleted from a comparison of the results between tooth germ with tumor tissue and tooth germ alone.

Eyes were checked periodically and the quality of each transplant's growth recorded and graded. Excellent growths were those that almost completely filled the anterior
chamber by 60 days, did not regress, and retained the morphology of the teeth. Transplants were graded from this level down to poor and negative growths. Those that grew for a time and then resorbed were considered poor. When tissues could not be observed in the anterior chambers following transplantation or they resorbed completely, these were considered negative growths. The growth of transplants was recorded in the following manner: ++++ excellent, +++ good, + fair, + poor, and — negative.

Recovered transplants were formalin-fixed and decalcified when necessary. Tissues were sectioned at 5-7 μ and stained with hematoxylin and eosin for histological study.

**Table A. Quality and Number of Takes**

| Growth | Tooth germ with tumor | Tooth germ without tumor | Totals |
|--------|-----------------------|--------------------------|--------|
| ++++   | 63 (44.7)             | 9 (27.3)                 | 72     |
| +++    | 42 (43.4)             | 28 (26.6)                | 70     |
| ++     | 28 (38.5)             | 24 (23.5)                | 62     |
| +      | 20 (28.5)             | 26 (17.5)                | 46     |
| —      | 35 (32.9)             | 18 (20.1)                | 53     |
|        | **Totals**            | **115**                  | **303**|

Expected numbers written in brackets.

X² with 4 degrees of freedom = 34.45.

P < 0.001.

**RESULTS**

The primary purpose of these studies was to determine the effect of this tumor tissue on growth and development of tooth germs when these tissues were transplanted together to the anterior eye chambers of guinea pigs.

When transplanted alone in the present experiments, this glioblastoma multiforme grew very slowly at first, but by 30-40 days it began to grow more rapidly. By 75-90 days its expansive growth usually ruptured the corneas. Figure 1 shows the tumor just rupturing the cornea 85 days following transplantation in one of the control series of the present experiments. Figure 6 shows a histological section of the tumor as seen grossly in Figure 1. This can be compared with Figure 5, which shows a histological section of the original tumor used for these experiments upon recovery from the brain of a guinea pig 70 days after transplantation.

The following biological events took place when tooth germs were transplanted alone in the present experiments. After a latent period of 4 or 5

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*Discarded animals not included in above table: 25 sick, died, or lost—with tumor and tooth germ; 22 eye infections—with tumor and tooth germ; 11 sick, died, or lost—with tooth germ; 9 eye infections—with tooth germ.*
Fig. 1. Anterior chamber transplant of tumor used as control for a series in the present experiments after 85 days. It is just rupturing the cornea.

Fig. 2. Anterior chamber transplant of tooth germ from a 30-day guinea pig embryo 65 days after transplantation. Control for series 17 in the present experiments.

Fig. 3. Anterior chamber transplant of tumor and tooth germ from 30-day guinea pig embryo 65 days after transplantation. Same series as above.

Fig. 4. Represents Figure 3, 125 days after transplantation.

Key to Symbols

D dentin
E enamel
G giant cells
I pigmented cells of iris
O odontoblasts
P pulp
T tumor
Bv blood vessels
OD osteodentin
Fig. 5. Histological section of the original tumor used for these experiments recovered after 70 days from the brain of a guinea pig. Original magnification x165.

Fig. 6. Histological section of the tumor shown in situ in Figure 1, after recovery from the host. Original magnification x165.

Fig. 7. Histological section of tumor 335 days after it was transplanted with a tooth germ. Tissue shown is approximately the entire amount of tumor recovered. Original magnification x165.

Fig. 8. Shows degenerating tumor tissue in contact with tooth transplant 76 days after transplantation. Original magnification x165.
FIG. 9. Shows pulpal area of transplanted control tooth germ 65 days after transplantation. Original magnification x165.

FIG. 10. Shows pulpal area of tooth germ transplanted with tumor 65 days after transplantation. Original magnification x165.

FIG. 11. Entire tooth germ transplant partially shown in Figure 10, and grown with tumor, for comparison with Figure 12.

FIG. 12. Represents tooth germ grown alone, partially shown in Figure 9. Original magnification of Figures 11 and 12, x19.
days, the transplants became attached to the irises by connective tissue cells and were then vascularized. Differentiation, growth, and development were usually rapid and continuous until mature calcified tooth structures were formed. After this, there was a gradual decrease in vascularity. The pulpal areas of such transplants also became less vascular and in addition their cellularity diminished. An osteoid revision followed these changes. It began within the pulps and extended to or involved adjacent dentine so that its customary tubular appearance was finally lost. The rate and extent of this osteoid revision varied, but usually it was initiated from 30-60 days following transplantation and has been extensively reported. Figure 2 shows a transplant of a tooth germ in place 65 days after it was placed in the anterior chamber. Histological sections are shown in Figures 9 and 12, and here the characteristic osteoid revision, avascularity, and acellularity are demonstrated.

The major observation in the present experiments was on enhancement of growth and development of a significant number of tooth germ transplants that were transplanted with the tumor. When both tumor and tooth germs survived transplantation together and maintained a contiguous relationship, development of the teeth took precedence over the tumor. This resulted in the formation of greater amounts of enamel and dentine. At such times, the tumor tissue also grew for a while, but by 100-125 days it had usually disappeared entirely. When transplanted with tooth germs, the tumor never seemed capable of growing expansively enough to rupture the corneas although the developing tooth sometimes did. Figure 7 shows a histological section of a tumor taken from an anterior chamber after 335 days with a developing tooth. This tooth germ transplant filled the anterior chamber by 90 days and maintained itself with an active blood supply for the ensuing period. The accompanying tumor after growing for awhile, resorbed and only a remnant about 2 mm. square was recovered. Continued growth of a tooth transplant in the presence of tumor tissue is demonstrated by Figures 3 and 4. Figure 3 shows the transplanted tissues after 65 days and Figure 4 indicates their condition after 125 days. These were from the same series or group from which Figure 2 was taken. In both instances, 30-day guinea pig embryonic litter mates supplied the tooth germs which were precursors of the third mandibular cheek tooth. Comparative difference in size in a control transplant and one transplanted with tumor tissue are shown in Figures 11 and 12. Figures 9 and 10 show the histological differences in the pulpal areas. Figure 8 shows a small bit of degenerated tumor tissue beside a tooth transplant after 76 days.

Pulpal areas of these transplants never appeared to be invaded by tumor cells when the tooth germ transplants remained intact and were not dam-
aged during transplantation. At times giant cells that closely resembled gitter cells were found within the pulp. Pulpal areas failed to become avascular and acellular as they did in control experiments with tooth germs. Pulpal cells remained young-appearing and blood vessels were more numerous than in control transplants with tooth germs. Odontoblasts remained in their normal position along the periphery of the pulp, and they did not disappear from the pulp as early as they did in control transplants.

Cells of the inner enamel epithelium, the ameloblasts, appeared to remain active and closely packed together for longer periods than those in the control animals. This continued activity resulted in the formation of more enamel than was found in the controls.

DISCUSSION

The ability of tooth germ transplants to survive and outgrow this tumor when both were simultaneously transplanted to a location where this tumor was known to survive and grow is in sharp contrast to the events taking place when certain faster growing tumors were used in pilot studies. The extent of this enhanced growth in a significant number of instances was greater than the expected growth and compared favorably with growth obtained in guinea pig female castrates.7

The biological behavior of the tooth germs and of this human neoplasm when transplanted together to the same location is of interest. Apparently growth potentialities of organ anlagen such as the teeth can be increased by transplanting them with certain transplantable neoplasms of approximately equal growth rates. The lack of a significant lymphocytic response by the hosts indicated that these tissues were compatible when transplanted together. Over the long periods of the present experiments, the tumor cells, after stimulating endothelial cells to proliferate and form capillaries or vascular channels, may never have had an opportunity to use these vessels entirely for their own growth and development. Furthermore, the subsequent rapid growth of the tooth germs apparently crowded out the tumor, leaving no space for it to grow.

It is difficult to compare the results obtained in these studies with the investigations of others because little has been done in this particular area of experimental biology. The nearest approach to a study of this type was the work of Browning,' where with different strains of mice he transferred alien tumors with homotransplants of embryonic tissues. When a tumor had the power of autonomy, he observed that growth of the embryonic tissue transplants was enhanced. The main point was that growth of embryonic tissues continued until that state of development normally attained
at birth was reached. In the present experiments, however, growth of tooth germ transplants was such that they often attained and sometimes exceeded the size of their in situ counterparts in adult hosts. Perhaps his (Browning's) results might have been different if it had been possible to transplant whole organ anlagen with tumor tissue such as was done with tooth germs in the present experiments. It is quite conceivable that tooth germs as organs are organized with a greater determination for growth than mere groups of tumor cells. This seemed especially true since both tumor and tooth germ transplants were initially about the same size and both had similar growth rates as indicated in these experiments.

It has been noted that congenital anomalies of teeth are the results, directly or indirectly, of metabolic insults occurring during embryonic growth. Ameloblasts, the highly specialized cells of the enamel organ, are greatly affected by environmental changes. The effect of prenatal influences on growth and development of the teeth has been sparsely investigated. Some information is available concerning factors or conditions that may retard or interfere with differentiation, growth, and development of the anlagen of the teeth, but there is little basic information about factors that are capable of augmenting their development. It is largely for this reason that this communication and a previous one may be of interest.

Basic metabolic machinery is a common property of all mammalian cells, and interruption of metabolic processes is potentially equal for all cells. However, tissues may vary in their responses to changes or alterations in the availability of metabolites. Such responses are an indication of differences in their dependence or vulnerability in a direct or indirect manner to oxygen or glucose deprivation. While most observations relative to growth and development of teeth have been on a negative side, very little information is available where environmental conditions are such that growth is enhanced instead of being interfered with. In the present experiments, conditions favored the enhancement of growth of the tooth germ transplants.

SUMMARY

When these human tumor and guinea pig tooth germs were transplanted together to the anterior eye chambers of guinea pigs, growth of the tooth germs and not the tumor was enhanced if these tissues maintained a contiguous relationship. Control transplants of tooth germs never attained the size of their counterparts which were transplanted with this human glioblastoma multiforme. Tumor tissue capable of rupturing the anterior chambers by expansive growth from 75-90 days after transplantation, when transplanted alone, did not do so when transplanted with tooth germs. At
times, however, the continued growth of the tooth germ transplants caused
a rupture of the corneas, a fact which did not ordinarily happen with these
single transplants. Tumor tissue survived for varying periods but usually it
was finally resorbed when in the presence of a transplanted tooth germ.
Enhancement of growth in these experiments was comparable to the
augmented growth obtained in guinea pig female castrates.

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