THE MANNER OF GROWTH OF FROG CARCINOMA, STUDIED
BY DIRECT MICROSCOPIC EXAMINATION OF LIVING
INTRAOCULAR TRANSPLANTS*

BY BALDUIN LUCKÉ, M.D., AND H. SCHLUMBERGER, M.D.
(From the Laboratory of Pathology, School of Medicine, University of
Pennsylvania, Philadelphia)

PLATES 23 TO 26

(Received for publication, June 20, 1939)

The characteristics of cancer growth have hitherto been studied chiefly
by histologic methods, that is in fixed, sectioned, and stained material.
The recent development of slit-lamp microscopy makes it now possible to
observe the habit of growth of tumors while living. Carcinoma of the
kidney of the leopard frog (1) has proved an admirable material for this
purpose. Bits of these tumors are implanted into the anterior chamber of
the eye, where they soon establish themselves, where details of form of the
growing tumor as well as arrangement of constituent cells can be directly
observed through the thin transparent cornea by means of the slit-lamp
microscope (2, 3), and where manner and rate of growth can be recorded
objectively by photograph. There is at present no other method which
permits as continuous observation of living tumor, and which gives as
vivid and as objective a picture of its manner of growth and progress.

In the present paper are reported the results of several series of related
experiments, dealing, first, with the characteristics of growth of homologous
transplants in the anterior chamber, second, with the fate of transplanted
normal kidney, and, third, with the growth of carcinoma transplanted in
the vitreus.

Suitability of Anterior Chamber for Tumor Transplantation

The suitability of the anterior chamber for tumor transplantation appears to have
first been recognized by van Doormaal (4), who in 1873 reported the results of his studies
on intraocular implantation of various homologous tissues into dogs and rabbits. Of
the transplants, that of mucosa of lip grew particularly well and developed into a “tumor-
like” mass. This finding led him to suggest the eye as a site likely to be favorable for

* This study has been aided by a grant from the International Cancer Research Foundation.

257
transplantation of benign and malignant neoplasms. Within the next few years three
investigators made use of this method, inoculating the anterior chamber of rabbits for
various purposes. Goldzieher (5) implanted several kinds of normal tissues; while some
grew for a considerable length of time, none showed tendency to form tumors. Leopold
(6) attempted to test Cohnheim's hypothesis of the origin of tumor from embryonal
rests; he transplanted fetal as well as adult cartilage; the latter was promptly resorbed,
while fetal cartilage grew well, and sometimes attained a mass several hundred times
that of the original implant. Leopold concluded that true tumors, chondromas, did
sometimes develop from such transplants; he regarded his results as experimentally
supporting Cohnheim's hypothesis. This view was not shared by Zahn (7); his implants
of fetal cartilage never became neoplastic and usually regressed after a variable period
of growth. Zahn in other experiments was the first to attempt intraocular transplanta-
ction of an actual tumor, a human hyaline chondroma; when, 80 days afterwards, the
animals were sacrificed, nearly complete resorption of the tumor had occurred.

The promising new method of intraocular transplantation seems now to have fallen
into disuse so far as studies of tumor problems are concerned. It was not until 1912 that
it was reintroduced by Ruben (8), who in an excellent paper pointed out its advantages
anew; he successfully transplanted Jensen's rat sarcoma into rats, but failed to obtain
growth when he used rabbits. Happe (9) transplanted a round cell sarcoma of rabbits
into different parts of the eye of homologous species; he obtained best results when small
bits of implants were inoculated into the anterior chamber; cell suspensions proved less
satisfactory. Hegner (10 a) and his associate Keysser (10 b) inoculated suspensions of
several types of mouse sarcoma and carcinoma into the eyes of mice, rats, guinea pigs,
and rabbits; in the homologous species rapid growth and occasional extensive metastasis
were obtained; in heterologous species the mouse tumors could be grown usually for two
or three passages. In another series, human sarcoma and carcinoma were inoculated
into rats' eyes; the carcinomatous transplants retrogressed, but in several cases growth
of sarcoma occurred and even retransplantation appeared to be successful. These in-
vestigators demonstrated that the eye is a suitable habitat for heterologous tumors.
However, Woglom (11) was unable to substantiate their claims; while suspensions of
the Crocker mouse carcinoma 180 readily took in mice, entirely negative results were
obtained when implantations were made into rats.

In more recent years, Brown and Pearce (12) have successfully transplanted their
rabbit sarcoma into the eye of homologous species. Moretti (13) has transplanted a
spindle cell mouse sarcoma into mice but failed to grow it in rats; he also attempted
without success to transplant human adenocarcinoma into the eyes of dogs (14). A rat
sarcoma has been transplanted into different parts of the eye of rats by Magnusco (15).
Gyotoku (16) has used Kato's rabbit sarcoma with a high degree of success; nearly all of
the transplanted tumors metastasized to internal organs. But he invariably failed to
obtain growth of a rat sarcoma in rabbits' eyes, though this tumor took readily in homol-
ogous species. Gyotoku describes in full the experiments of two Japanese investigators
whose work is published in inaccessible journals; Mori (17) successfully transplanted
Fujinami's chicken sarcoma into chicken; filtrates did not produce growths; Nagayo (18)
had negative results with mouse carcinoma transplanted into rats. Two other Japanese
investigators, Nishi (19) and Takayasu (20), have more recently reported on successful
homologous transplantation of rabbit sarcoma.

Lately Smirnova (21), in a brief paper, has given the results of his extensive experi-
ments on a variety of tumors implanted into the anterior chamber of several species of laboratory animals. Human mammary cancer inoculated into white rats, guinea pigs, and rabbits grew well up to 4 months; Ehrlich's mouse sarcoma grew up to 6 months in rats and rabbits, the Brown-Pearce tumor and a rat sarcoma grew well in white rats. At the present time, Greene (22) is studying the behavior of a uterine adenocarcinoma of rabbits transplanted in the eyes of homologous as well as heterologous species. In his homologous transplantation experiments the tumor has been observed through 12 serial generations. These experiments have shed much light on the nature of the factors determining the characteristics of transplanted tumor. His is by far the most thorough investigation of a particular tumor by the method of intraocular implantation. He has also given a preliminary account of successful heterologous transplantation of human and other mammalian tumors (23).

From this survey of the literature it will be apparent that the eye has been used as an inoculation site for tumor rather more frequently than is generally recognized. Nearly all of the investigators have emphasized the ease with which intraocular transplants of tumors become established; indeed, the aim of most of the experiments was to test the suitability of the eye for tumor inoculation in various homologous and heterologous species. The growth of the transplanted tumors has usually been studied only by means of excised and sectioned material, though a few investigators have made some observations of the living tumor. The development of slit-lamp microscopy has opened a new field for studying more effectively the characteristics of tumor growth, and this has been the aim of our experiments.

Methods

Unselected adult leopard frogs (*Rana pipiens*) were used. All of the tumors for transplantation were typical large, invasive adenocarcinomas of the kidney obtained from other frogs of the same species. Healthy appearing portions from 13 such tumors were cut into small bits, averaging 1 to 2 mm. in diameter, and transplanted immediately afterwards into the anterior chamber of 146 frogs; similar fragments from 2 other tumors were implanted in the vitreus of 31 frogs. Bits from normal kidneys of 4 non-tumor-bearing frogs were implanted in the anterior chamber of 76 animals.

Operations, and photography of transplanted tumors, were performed under anesthesia. The frogs were placed in a jar through which ether vaporized by air was slowly pumped with a rubber bulb. Within 5 minutes the animals were usually anesthetized sufficiently to permit operation. A wad of cotton was now inserted into the mouth to cause the eyes to protrude and to immobilize them to some extent. The frog was next

---

1 The orbit of the frog is not separated from the oral cavity by a membranous partition. Hence the eye can be made to protrude from the orbit by pressure from the mouth cavity. This greatly facilitates operative procedures; however, care must be taken not to exert excessive pressure upon the eyeball, as by using too large a wad of cotton, lest intraocular pressure be too greatly increased and the iris later prolapse through the wound.
wrapped in moist gauze, placed on its right side, and firmly held in this position by stout pins crossed over the animal. Thorough rinsing of the left eye with 2 per cent boric acid solution, followed by amphibian saline, completed the preoperative procedures.

For inoculation of the anterior chamber, the upper eyelid was retracted and an incision, 2 to 3 mm. in length was made with a small knife along the upper border of the sclerocorneal junction, care being taken not to cut into the iris, for the resulting profuse hemorrhage may prevent successful transplantation. The unavoidable oozing of aqueous tumor tended to cause gaping of the wound, through which the transplant was quickly inserted with delicate long-pointed forceps. The transplant afterwards was moved to any desired part of the chamber by gently stroking the surface of the cornea with a rod. The cotton plug was removed, thereby relieving intraocular pressure, and the animal placed in a glass jar with moist cotton. Here it remained for about a week before transfer to the aquarium where it was kept as described previously (1). Precautions for sterility were observed throughout the operation and care was taken to keep knives sharp and forceps ground to long needle-like points. With these precautions, infection was rarely encountered and prompt healing took place.

Photographic records of the transplants were made periodically. The anesthetized frog, wrapped in wet cotton, was mounted in front of the slit-lamp on a board between adjustable side bars. The eye was held open by wedging a pledget of cotton between lower lid and orbital rim. For photography at magnification of a few diameters, the entire eye was illuminated evenly by using a wide slit. For higher magnification a narrow beam was projected onto the area to be photographed. Drying of the cornea was prevented by occasional rinsing with water; no heat filter was necessary. Low power photographs were taken directly with a Leica camera, equipped with extension tubes; for greater magnification, the camera was mounted on a mononuclear microscope tube provided with the proper objectives. Exposure varied from 1 second up to 20 seconds, depending on the intensity of illumination required. The adjustability of the apparatus, both as to movements and degree of illumination, greatly facilitated the making of the photographic records.

Direct observations of the transplants were made on unanesthetized animals through the slit-lamp microscope. The frogs were mounted as for photographing, but without retracting the lower lid and nictitating membrane; usually they voluntarily opened their eyes after a few moments and remained sufficiently quiet, so that, with occasional re-adjustments of illumination and focus, even prolonged observations were possible. The procedures described permitted observation at magnifications up to 100 diameters, approximately. Growth of transplants was usually followed over a period of 2 or 3 months, and in selected cases for over 6 months.

In order to compare the direct observations of the living tumors with the appearance obtained in histologic sections, and for purpose of examining the interior of the transplants, eyes from animals which had died or which were sacrificed were fixed in Susa solution, embedded in celloidin, sectioned at different levels, and stained with hematoxylin-phloxin, or with Masson's trichrome mixture.

Aside from examination of the inoculated eyes, complete postmortem examinations were performed, with particular attention to the kidneys. Groups of control frogs, comparable in number to the experimental series, were maintained for the purpose of evaluating the development of renal tumors expected from the results of previous transmission experiments. This phase of the study will be reported on at a later date.
Over 50 per cent of transplants from 10 of the tumors grew readily, those from 3 other tumors made but little progress and were soon absorbed or became fibrosed; the cause for this difference was not ascertained. In the first group, successful transplantation ranged from 10 per cent in the case of one of the tumors to 100 per cent in 2 tumors, the average percentage of takes for the entire series being 53.9 (Table I). In general, therefore, the anterior chamber is a favorable site for transplantation of the frog carcinoma.

<table>
<thead>
<tr>
<th>Designation of transplanted tumor</th>
<th>Number of animals used</th>
<th>Takes per cent</th>
</tr>
</thead>
<tbody>
<tr>
<td>555</td>
<td>19</td>
<td>31.6</td>
</tr>
<tr>
<td>558</td>
<td>14</td>
<td>50.0</td>
</tr>
<tr>
<td>566</td>
<td>18</td>
<td>66.6</td>
</tr>
<tr>
<td>567</td>
<td>10</td>
<td>10.0</td>
</tr>
<tr>
<td>602</td>
<td>19</td>
<td>42.2</td>
</tr>
<tr>
<td>644</td>
<td>5</td>
<td>20.0</td>
</tr>
<tr>
<td>602 A</td>
<td>10</td>
<td>90.0</td>
</tr>
<tr>
<td>665</td>
<td>10</td>
<td>100.0</td>
</tr>
<tr>
<td>672</td>
<td>6</td>
<td>100.0</td>
</tr>
<tr>
<td>708</td>
<td>4</td>
<td>50.0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>115</strong></td>
<td><strong>53.9</strong></td>
</tr>
</tbody>
</table>

The appearance of the transplant as seen through the low power of the slit-lamp microscope immediately after the operation is shown in Fig. 1. The pale color of the tumor contrasts sharply with the dark background of the iris. The surface when viewed with low magnification appears smooth, though with greater magnification minute ragged tags of tissue are seen; within 2 or 3 days these are absorbed and the surface becomes entirely regular in outline. This condition may persist for several days or as long as a month, depending on the temperature of the environment and the growth energy of the particular strain of tumor. During the period of lag no noticeable exudative reaction to the transplant occurs, and if infection is avoided, none develops later.

The transplant generally becomes attached within a week to the iris, lens, or more rarely the cornea; at first the attachment is so light that even slight pressure upon the eyeball may cause dislodgment. This may occur during the handling incident to observation or photography. However, dislodgment does not appear to disturb further development, and reattachment soon takes place. The transplants which become fixed to the inner margin of the iris, i.e. to the pupillary region, generally show much better growth than those which have lodged near the periphery. This position is probably more favorable because of the greater depth of the anterior chamber in the pupillary...
region, and the consequently greater gaseous and other exchange between transplant and surrounding aqueous tumors.

Even before growth becomes evident there usually is a distinct difference between those transplants which will persist and those which will vanish; the former have a certain translucency, the latter become increasingly opaque. The difference is quite similar to that existing in tissue culture between healthy and dying explants.

Observations of many transplants has led to the conclusion that the form which the growing tumor assumes depends on its immediate physical environment. Where the tumor grows out in the midst of the aqueous humor, unimpeded by solid tissue, its habit of growth is characteristically tubular or papillary. If, however, the tumor grows in contact with a firm even surface such as lens or cornea, then the form of growth is entirely different; broad membranes are formed which extend along and cover the surface. If, on the contrary, contact is made with loose distensible tissue, such as iris, a third type is assumed; the tumor extends downward forming bizarre shaped tubules and alveoli within the preexisting stroma of the invaded tissue. The development of the first two types of growth may be followed step by step by slit-lamp microscopy; for study of the third type histological sections are required.

**Tubulo-Papillary Manner of Growth.**—This type is the most common and conspicuous. First, the surface of the transplant becomes increasingly translucent, due to outgrowth of new cells. Then small knob-like buds form which gradually elongate to solid, straight, cylinders usually having blind, bulbous terminal portions (Fig. 2). As growth proceeds, more and more of such projections develop, giving to the surface of the tumor a villous or papillary character (Fig. 3).

In its early stages at least, each projection is exclusively composed of tumor cells, and has neither lumen, nor fibrovascular stalk. With elongation, the cylinders tend to become somewhat curved, and most of them gradually acquire a central lumen, slit-like at first, later more conspicuous, or even cystic (Fig. 4). The tubules increase in complexity by repeated dichotomous branching, and, as growth advances still further, numerous sac-like outpouchings arise from their wall, the arrangement being quite comparable to that of alveolar ducts and alveoli (Fig. 5). The tridimensional appearance obtained by direct examination of the living tumor gives a far more complete appreciation of the extent by which such lateral outpouching increases mass and surface than does the bidimensional impression gained from the histologic section (Fig. 6). As a rule, only the coarser solid outgrowths acquire a vascular stalk (Fig. 7), and this usually as late as several weeks after transplantation. Indeed, the size of the transplant may increase many fold without vascularization; the currents of fluid in the anterior chamber appear to be sufficient for maintenance and multiplication of the tumor cells.

**Membranous Surface Growth.**—In contrast to the highly differentiated form of growth just discussed is the indifferent membranous growth which the proliferating tumor assumes when it comes in contact with firm surfaces. Such membranes are best seen
and attain greatest extent when tubular outgrowths reach a large, smooth, non-yielding surface, such as lens or cornea. To such the tubules become adherent; afterwards their proliferating cells no longer form a cylinder, but spread as an undifferentiated layer over the surface.

In the living implant, the membranes in their early stages appear as delicate filmy sheaths with indefinite boundaries, but as cell multiplication progresses, they thicken, and acquire sharply defined margins (Fig. 8). In corresponding histologic sections, one sees that the film consists at first of a single layer of flattened thinly spread cells (Fig. 9); at later stages cellular carpets, several layers in depth, develop (Fig. 10).

Once membranous growth has started, it tends to extend laterally as long as the creeping cells meet with no obstacle; where, however, irregularities of surface are encountered, or where cell multiplication is particularly active, protrusions may be found, and groups of two or three tubules may thus secondarily arise from the basement layer (Fig. 11). In addition to these extensive membranes small isolated islands of flat growth often form on the surface of the transplants, and on the iris.

As we shall show more fully in the following paper (24), the chain of events discussed, i.e. tubular outgrowth which becomes membranous as contact is made with firm surfaces, is extraordinarily similar to the growth regularly encountered when this tumor is explanted in plasma cultures.

Acinar Invasive Growth.—The third type of growth is observed when the proliferating mass, either tubular or membranous, invaginates into the loose spongy tissue of the iris. Now a close return to the original adenocarcinomatous form occurs. The invading cells become arranged as bizarre shaped tubules usually with a conspicuous lumen, and always with an external stromal support (Fig. 12). There seems to be no doubt that it is this support, as well as the loose structure of the invaded tissue, which determines the character of the growth. In many cases all three types of growths are represented: tubulopapillary into the fluid of the anterior chamber, membranous over lens or cornea, and invasive acinar in the iris.

Rate of Growth of Transplanted Tumor.—Photographs made periodically of the transplanted tumors furnished objective records of their form and size. A representative group of six such records is shown in Figs. 13 to 18.

The first photograph (Fig. 13) illustrates a relatively early stage of growth of a tumor which is attached to the lower margin of the iris and covers a portion of the lens. The contour, which was smooth and round during the period of lag, has become irregular and knobby, and from the free surfaces a dozen or more villous projections extend. A week afterwards the mass had increased several fold and covered the major part of the lens; some of the numerous projections had extended to the opposite margin of the iris. After this rapid period of growth, progress was more slow; but as the succeeding photographs show, the surface projections became more complex and coarser, the tumor as a whole acquired a more massive appearance, and spread over the iris which it infiltrated.

While the extreme irregularities of surface of these tumors make it impossible to measure their rate of growth in physical terms, the photographic records do give a good idea of the comparative rates of growth of different
transplants. In general it was found that growth followed a similar pattern, which was independent of the strain of tumor, external temperature, or season. Following a variable period of lag, there came a period of gradual tubular outgrowth, to be succeeded rather abruptly by a relatively short period of rapid growth, after which the increments gradually became smaller and smaller. The range of the characteristically rapid increase in size during a period of 9 days is illustrated by 3 different transplants from the same tumor (Figs. 19 to 24).

The interrelated effects of season and of temperature require further investigation. In the vivarium which housed the frogs, no actual hibernation occurred, though the animals became sluggish and refused food during the colder months when the temperature of the air ranged around 15°C., and that of the running water dropped to 5-8°C. While transplants became established at all seasons, growth was very slow during the winter. No decisive differences, however, were noted between transplants made during the spring, the period of the frog's greatest activity, and autumn when activity and metabolism decline. In fact, with some strains of tumor more progress was apparent in autumn than in the spring and summer months. Thus, the photograph shown in Fig. 14 was made 41 days after transplantation in October; the size attained compares favorably with that of another tumor, the appearance of which, 78 days after transplantation at the end of March, is shown in Figs. 19, 21, and 23.

Ultimate Fate of Transplanted Tumor.—The interior of the original graft usually becomes more or less fibrous, while the cells at the periphery proliferate and assume the various types of growth discussed. In occasional transplants, no fibrosis occurs, and the original acinar structure is maintained; in such cases the transplants enlarge not only externally but centrally.

After the transplanted tumor has attained considerable size and occupies most of the anterior chamber, backward extension into the posterior chamber and occasionally into the lens or vitreus may take place; no extraocular extension was observed. In but few cases has it been possible to follow the fate of such large tumors (most of them were used for other purposes); in them, gradual decline in size, regression, and increasing fibrosis appeared to be the general rule, once the size of the tumor exceeded its requirements for growth. The relatively small and confined space in the anterior chamber, and the progressive increase in intraocular pressure with resulting circulatory disturbances, seemed to be important limiting factors, for in the roomier vitreus some tumors attained much greater mass.
Transplantation of Normal Kidney into the Anterior Chamber

In a preceding paper evidence has been presented which led to the conclusion that the kidney tumor of the leopard frog is caused by an organ-specific (nephrotropic) virus (1). If, therefore, it were possible to transplant normal kidney into the anterior chamber, a method would be available for directly observing the changes of this tissue following inoculation with tumor-inducing agent. Unfortunately not a single transplant of normal kidney of a total of 76 became established. Though most of them became vascularized more readily than did the tumor transplants, no outgrowth developed in any; the renal tubules soon deteriorated and were replaced by fibrous tissue. It is of some interest to record the persistence of certain glomerular components, namely flat cells which cover the otherwise fibrous remnants of tufts and their capsules. Their appearance in a transplant 204 days after inoculation is shown in Fig. 25.

Transplantation of Tumor into the Vitreus

The vitreous humor differs physically from the aqueous in its gelatinous consistency and larger amount; chemically it resembles the aqueous excepting for the presence of additional proteins (25).

The operative procedures for transplanting bits of tumor were similar to those described for the aqueous, except that the initial incision was made slightly posterior to the sclerocorneal junction; in this operation severe bleeding is quite apt to occur. The transplants grew well, and in a manner entirely similar to those in the anterior chamber. They spread as broad membranes over the retina and the posterior surface of the lens, and extended as tubules and papillary projections into the vitreus (Fig. 26). Although such tumors cannot directly be observed, they may attain considerably larger size (Fig. 27) than they do in the anterior chamber, and hence serve more readily for the propagation of a given strain.

SUMMARY

The adenocarcinoma which commonly occurs in the kidney of leopard frogs has been transplanted into the anterior chamber of the eye where its growth characteristics have been studied by direct observation with the slit-lamp microscope. Such observations have been amplified by photographs taken at intervals to furnish permanent and objective records of the mode of development and progress of the growths, from earliest to advanced stages.

The fifteen tumors which furnished the transplants were typical large
invasive adenocarcinomas having the usual irregular and apparently anarchic arrangement of their component tubules and acini. However, the transplanted tumors developed according to definite and well defined structural patterns, their type depending on the immediate physical environment. Three such morphogenetic patterns were observed.

Unimpeded outgrowths into the aqueous tumor characteristically assumed a tubulo-papillary arrangement; the earliest formation consisted of solid, purely epithelial cylinders, many of which at later stages acquired a lumen and thus became tubular; generally only the coarser projections developed vascular stalks. Further growth was made by repeated branching, and lateral outpouching of the tubules. Where the tumor grew in contact with firm, even surfaces, such as lens or cornea, differentiation was lost and broad membranes formed which gradually spread over the surfaces; secondarily, new cylindrical or tubular processes arose from such indifferent cellular carpets. Where the tumor made contact with loose tissue such as iris, it invaded this organ, and, supported by the stroma, assumed an acinar pattern quite like that of the original adenocarcinoma of the kidney. All three types of growth were sometimes found coexisting in different portions of the anterior chamber.

The rate of growth of transplanted tumors was followed by photographic records taken periodically. A fairly constant mode of progress was noted: after a variable period of lag and a period of gradual outgrowth, there followed rather abruptly a short period of rapid growth, after which growth increments gradually became smaller. Variations in growth rate due to season and to temperature were evident, but further experiments are required to evaluate the part played by these factors.

Attempts to transplant normal kidney in the anterior chamber were unsuccessful.

The manner of growth in the vitreus was found to be similar to that in the anterior chamber.

The factors that determine the manner of tumor growth are as yet imperfectly understood. Evidence is however accumulating to the effect that neoplastic growth is not as anarchic as is suggested by histologic sections of some tumors (26). The present experiments support the view that cancers are much more responsive to the laws governing growth and organization than is generally supposed.

BIBLIOGRAPHY


8. Ruben, L., Arch. Ophth., 1912, 81, 199.


17. Mori, S., cited by Gyotoku (16).


EXPLANATION OF PLATES

Figs. 1, 5, 7, and 13 to 24 are unretouched photographs of living tumors; the remainder are sections which were stained with hematoxylin and phloxin. All magnifications are approximate. All of the figures were photographed by Mrs. Miriam R. Barrett.

PLATE 23

Fig. 1. Transplant immediately after operation to show size in relation to that of pupil. Note rounded contours of transplant. × 9.

Fig. 2. Early outgrowth, 17 days after transplantation. Surface of the transplant shows several knob-like buds and a straight, solid cylindrical projection with a bulbous termination. × 30.

Fig. 3. A more advanced stage of outgrowth, 31 days after transplantation. Numerous tubules project into the aqueous humor giving to the surface of the tumor a villous character. × 20.

Fig. 4. A section of the tumor shown in preceding photograph. The tumor is attached by a broad fibrous stalk to the capsule of the lens. From its surface project numerous club-shaped structures; some of these are solid, others have a narrow cleft-like lumen, in a few the lumen is distended; none have a central supporting stalk; many show branching. Laterally the tumor extends as a thin membrane over the iris.

Fig. 5. Appearance at high magnification of a single tubular outgrowth, 82 days after transplantation. There are numerous lateral outpouchings which cause a strong resemblance to an alveolar duct of the lung with its sacculi. × 100.

Fig. 6. Histologic section of a tubule from the tumor shown in Fig. 5. The bidimensional appearance of the section gives no indication of the complex outpouching clearly to be seen in the photograph of the living tumor. × 100.

Fig. 7. A coarse papillary stalk having a well vascularized, loose, edematous stroma. × 130.

Fig. 8. A broad membranous growth (below) which has spread from the edge of the transplant (above) over the surface of lens (63 days after transplantation). × 20.
(Lucké and Schlumberger: Manner of growth of frog carcinoma)
PLATE 24

Fig. 9. The tumor has spread in a single cell layer over the lens capsule, beneath which the normal epithelium is to be seen. Above the membrane a tubular growth, which was attached to iris, extends into the aqueous humor. × 260.

Fig. 10. Two types of growth are represented. Over the posterior portion of the cornea a thick undifferentiated membrane has formed. Extending into the anterior chamber there are coarse papillary projections with a loose vascular stroma derived from the iris. × 110.

Fig. 11. A broad membrane which covers the capsule of the lens has in several places evaginated and formed short tubules. × 200.

Fig. 12. This section is from the tumor shown in Fig. 10 and exhibits a third type of growth, namely acinar formation in the loose stroma of the iris, which forms the external support of the acini.
PLATE 25

FIGS. 13 to 18. A series of photographs to illustrate the progressive growth of a transplanted tumor inoculated on Oct. 8, 1938. The dates of the photographs are Nov. 11, 18, 28; Dec. 13, 19, 1938; and Jan. 13, 1939. × 11.
(Lucké and Schlumberger: Manner of growth of frog carcinoma)
PLATE 26

Figs. 19 to 24. 3 different transplants from the same tumor photographed first on June 7 (Figs. 19, 21, and 23), and again after 9 days (Figs. 20, 22, and 24) to illustrate the amount of growth during this period of time. Date of inoculation: Mar. 22, 1938.

In Figs. 22 and 24, note the filmy membranous growth which has spread from the central tubular mass over the surface of the lens. × 9.

Fig. 25. Appearance of transplanted normal kidney; the renal tubules have completely disappeared, their place having been taken by hyaline fibrous stroma in which lie remnants of several glomerular tufts. The cores of the tufts as well as capsules of Bowman are covered by well preserved cells. ×200.

Fig. 26. Growth of tumor in the vitreus. The surface of the retina is lined by a tumor membrane from which project numerous short tubules and coarser papillary stalks. ×60.

Fig. 27. The entire vitreus is filled with a tumor growing in tubular and papillary arrangement. Date of examination is 147 days after inoculation.
(Lacké and Schlumberger: Manner of growth of frog carcinoma)