UTERINE ADENOMATA IN THE RABBIT

II. HOMOLOGOUS TRANSPLANTATION EXPERIMENTS

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PLATES 28 AND 29

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The occurrence and course of spontaneous adenomata and adenocarcinomata of the uterus in the rabbit and the successful transplantation of one of the tumors to animals of the same species were reported in a previous paper (1). Investigation of the transplanted tumor has been continued and extended in various directions with a view to determining the essential biological and pathological characteristics of the growth as compared with other transplantable tumors now used for experimental purposes.

Many spontaneous tumors have been reported in the rabbit but very few have been successfully transplanted to normal animals. With the exception of the Brown-Pearce epithelioma (2) all of the transplantable tumors have been sarcomata and with the further exception of Kato’s sarcoma (3) all were lost before any extensive experimentation could be carried out. During the past year, three different types of spontaneous rabbit carcinomata, in addition to the uterine tumor, have been successfully transplanted and are being studied in a similar manner to supply a broader basis for comparison and as additional agents for the investigation of cancer problems. It has been found that the uterine tumor, designated as H31, is manifestly different from other transplanted rabbit tumors and possesses certain potentialities which render the growth a more advantageous medium for the study of certain phases of the tumor problem.

The object of the present paper is to report the results of several series of interrelated experiments including: first, serial transplanta-
tion in normal rabbits; second, transplantation in rabbits with spontaneous tumors of the same nature; third, reinoculation of animals with a growing transplanted tumor; fourth, reinoculation of animals which proved refractory to an earlier attempt at transplantation and of animals in which transplanted tumors had completely retrogressed and finally, a series of experiments in which frozen and dried tumor material was introduced into normal uteri.

While this report must be limited to results obtained with the uterine tumor referred to above, comparative statements of results are based in part upon experience with other tumors. Details of these experiments will be reported later.

Materials and Methods

The technique of transplantation employed in the present experiments has been fully described elsewhere (1). Whole fragments of tumor tissue measuring approximately 1 mm. in diameter were used in transfers to the anterior chamber of the eye, while testicular inoculations were made with 0.3 cc. of a thick cellular emulsion.

The present report is based on a clinical and pathological investigation of the tumor through 12 serial generations in the anterior chamber of the eye and 6 serial generations in the testicle. Tumor tissue was transferred to the eyes of 23 normal males and 62 normal females while 95 males were used for testicular inoculation. The results of simultaneous inoculation of both testicles or of one testicle and an eye were studied in 5 animals. Transplants were also made in 8 females with spontaneous uterine tumors and 13 animals in which the tumor had failed to grow were reinoculated after periods of 35 and 69 days following the first inoculation. In addition, transplants were made in 3 animals in which a previously transplanted tumor was actively growing and in 13 animals in which the previously transplanted tumor had completely retrogressed.

Six animals were used in an attempt to induce neoplasia with dried frozen tumor material. Fresh tumor tissue was obtained from the eye of an animal of the 6th serial generation. The tissue was immediately frozen in dry ice and dried in a vacuum machine. The resulting powder was emulsified in water and injected directly into the uterine mucosa. The animals were killed 42 days after injection and sections of the mucosa were examined histologically.

Throughout the experiments particular attention was directed toward a thorough gross and histological postmortem examination, not only to follow the morphological characteristics of the tumor and to discover the presence or absence of metastases but, also, to determine the condition of those organs altered in spontaneous cases of the tumor. Pituitary glands were fixed in Susa's solution and stained by a modification of Mallory's aniline blue method, while routine
tissues were fixed in Petrunkevitch's solution and stained with hematoxylin and eosin.

**Serial Transplantation into the Anterior Chamber**

The results of serial transfer of tumor fragments into the anterior chamber of the eye are presented in Table I. Successful transplantation was effected in 82.8 per cent of all the animals used but varied from 33.3 per cent to 100 per cent in different generations. In early transfers the tissue was obtained from transplants varying from 2 to 3 months in age while in later transfers the interval between transplantations rarely exceeded 1 month.

The age of the transplant undoubtedly played a part in the greater incidence of takes but there is also evidence that adaptation to the environment of the anterior chamber was of considerable importance in this respect. Two experiments bear on this point. In one experiment tissue fragments from a growth in the testicle representative of

<table>
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<td>3</td>
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<td>33.3</td>
</tr>
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<td>8</td>
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<tr>
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<td>Feb. 24</td>
<td>6</td>
<td>83.3</td>
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<td>Mar. 3</td>
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<td>8b</td>
<td>&quot; 12</td>
<td>14</td>
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<tr>
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<tr>
<td>Total</td>
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<td>93</td>
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the 7th generation of the tumor were transferred to the anterior chambers of 14 animals and resulted in 38.5 per cent of takes. In a second experiment the transfer of fragments of another testicular growth of the same generation into the eyes of 13 animals resulted in 41.6 per cent of takes. These results are similar to those observed in the first part of the series under discussion, despite the fact that the tumor had been successfully transplanted for 7 generations, and indicate that the high percentage of takes in the latter part of the series was influenced by adaptation to the environment of the anterior chamber, rather than by adaptation to transplantation in general.

The regular increase in the incidence of takes was interrupted in August, 1937, and in July, 1938. A similar reduction in the number of takes during these months has been noted in the study of other tumors propagated by different routes of inoculation and has occurred to a much greater degree in the testicular series of the present tumor.

Latent Period.—A gradual decline in the period required before growth of the transplanted fragments became apparent was observed throughout the first 5 generations but, even after the 5th transfer, definite evidence of growth could only rarely be detected in less than 1 month. Abruptly after the 6th transfer this period was cut to 14 days and while, at the present time, signs strongly indicative of growth may be found in a shorter period, an earlier positive diagnosis of growth cannot be made.

The first indication of survival is a pinkish color change in the transplanted fragment without any appreciable increase in its size. At the present time, this can often be detected by the 6th day. The color change appears simultaneously in all parts of the fragment and increases slightly in intensity throughout the following week. In contrast, fragments that fail to grow become dull white in color and opaque. In recent transfers, minute pin point pinkish-white areas are frequently observed in regions where formerly no trace of the transplant could be seen. Such areas have been interpreted as resulting from the growth of cells dislocated from the graft during its passage through the chamber.

An increase in the size of the transplant can usually be detected by continuous observation during the 2nd week. This is facilitated by the transplantation of fragments with irregular outlines so that small changes in a given locus can be readily perceived. Comparative drawings taken at daily intervals, in such cases, show a slight but definitely detectable tendency toward the rounding out of sharp, angular irregularities which is usually apparent by the 8th day. This process continues and at the time of vascularization, the irregular outlines have disappeared and the transplant is round or oval in shape.

Vascularisation and Subsequent Growth.—The transferred tumor fragments
always become attached to a fixed part of the anterior chamber, usually the iris, within 12 hours of transplantation. Attachment occurs whether or not growth subsequently takes place and is evidently brought about by an exudative reaction on the part of the host. It is not associated with a connective tissue or vascular proliferation but is loose and may be disrupted by applying pressure along the corneal surface. Evidence of vascularization has not been detected before the 14th day and is frequently not observed before the 21st day. It should be noted, however, that stroma replacement and vascularization began along the area of attachment and the process may be of some duration before blood vessels can be seen in the gross on the exterior of the transplant.

In all but a single instance, fragments that have undergone the color change and increase in size previously described have eventually become vascularized. Vascularization is often delayed for as long as 3 weeks and in the interim the fragment may double its mass or remain without appreciable change. In exceptional cases, fragments have persisted without a detectable blood supply or increase in size for 2 months and then have become vascularized with subsequent rapid development into large tumors.

Vascularization is effected by a growth of vessels from the iris which permeate the graft in all directions and form a fine, complicated network about the growing edge. The appearance of the vascularized transplant is sufficiently characteristic to differentiate it from grafts of other tumors of different origin growing in the same environment. The tissue appears homogeneous and pale pinkish-white in color, except for the peripheral region which is of a deeper hue. Occasional minute greyish flecks are seen but the patterned arrangement of dark and lighter areas found in other growths is entirely absent. The tendency to form rounded masses with smooth clear-cut edges persists until infringement on the boundaries of the anterior chamber forces a change of shape.

**Eventual Fate.**—A large proportion of the animals used in these experiments were killed as soon as successful transplantation into the next serial generation became apparent, but others were held to determine the eventual fate of the growth.

The growth rate was increased after vascularization in all instances but varied in different generations and in individual animals. The period required for the complete filling of the anterior chamber varied from 45 to 105 days in animals of the same generation and, while the chamber was rarely filled in less than 60 days in the earlier transfers, complete replacement has been observed by the 20th day in recent generations. It should be noted, however, that in recent generations, growth invariably occurred in multiple foci and the resulting nodules coalesced so that the increase in growth rate was not as great as is suggested by these time relations.
After filling the anterior chamber, the growth underwent regressive changes in approximately 70 per cent of cases. The tissue became brown, granular and opaque and, eventually was entirely resorbed leaving no permanent damage other than large corneal scars and occasional synechiae. On the other hand, in the remaining 30 per cent of cases, growth continued, the cornea was invaded and the tumor protruded externally as a large fungating mass. Animals of this class have been killed for humane reasons, and hence there is no telling what their eventual fate would have been. Autopsy revealed the presence of metastases in two instances. One animal had been killed on the 184th day after transplantation and secondary growths were found in the regional lymph nodes, the lungs, the pancreas and the left ovary. In another animal killed on the 161st day, the regional nodes alone were involved.

As a rule, the more rapidly growing transplants undergo regression after filling the chamber, while those with a slower growth rate tend to invade and to extend to the outside. It seems probable that the regressive changes may be a direct result of an increased intraocular pressure incident to rapidly expanding growth in a confined space and leading to a progressive diminution of blood supply. The fact that, in the normal course of events, regression has never been observed before the entire chamber is filled is also suggestive in this respect. It is of interest in this connection that surgical interference with removal of a small part of the graft is almost invariably followed by regression.

No attempt was made in the present series of experiments to select animals to test the effect of different constitutional factors on the susceptibility to transplantation or on the eventual fate of the growth. Hybrids were used, for the most part, and no indication of breed differences can be obtained from the data. An analysis of the results on an age basis shows that while no significant variation in the susceptibility to transplantation occurred, the transplants grew more rapidly in young animals and after replacing the anterior chamber, regression rather than continued extensive growth was the rule. The percentage of takes was the same in males and in females and the subsequent fate of the tumor was not altered by the sex of the animal.

Histological Examination.—Histologically, the transplants obtained from animals of early serial generations showed an approximate duplication of the characteristics of the parental tumor (Fig. 1). There was an abortive attempt at the formation of acini which for the most part were composed of solid cellular masses and were without a lumen. Mitotic figures were not numerous and degenerative changes were rarely found. The stroma was abundant and myxoid in character.
In later generations, growth was more atypical and anaplastic (Fig. 2).

A tendency to form rounded masses was still apparent, particularly along the advancing edge of the tumor, but in other regions individual masses had coalesced to form solid sheets. In such regions the frequent occurrence of round areas of necrosis surrounded by concentrically arranged epithelial cells indicated the manner of growth. Stroma was sparse and poorly differentiated. Large cellular regions were separated by fibrous connective tissue extensively invaded by epithelial cells which formed abortive acini in some areas but grew, for the most part, in short strands and columns giving the tissue a marked resemblance to sections of scirrhouss carcinoma. The number of mitotic figures and the amount of necrosis were directly proportional to the rate of growth observed clinically.

Necrosis dominated the picture in animals killed while the tumor was undergoing clinical regression. Large circumscribed necrotic areas were distributed focally throughout the cellular regions and in older growths these regions were completely necrotic except for a narrow rim of intact cells surrounding vascular channels. On the other hand, epithelial cells enmeshed in fibrous tissue remained intact for a longer period of time and the eventual disintegrative changes proceeded through karyorrhexis rather than karyolysis as in the more cellular areas.

All structures of the anterior chamber with the exception of the lens were invaded and destroyed in the large fungating types of growth. The posterior chamber was frequently involved and was occasionally found filled with tumor, but extension through the sclera to retro-orbital tissues has not been observed.

Metastases were less cellular in structure than the primary growth and the connective tissue reaction was more marked (Figs. 3 and 4). The proliferation of fibrous connective tissue was so intense in the lung, particularly near the pleural surface, that serial sections were frequently required before the essential epithelial elements could be found. The resemblance to scirrhouss carcinoma was striking in these regions but in other organs parenchymal cells were grouped in atypical acinar arrangement and the stromal relations approximated those of the primary growth.

*Serial Transplantation in the Testicle*

Transplantation of the tumor into the testicle was successfully carried out from the 3rd, 4th and 5th serial eye generations and the growth obtained from the 4th eye generation has been propagated by continued serial transfer in two different lines of animals.

The results of serial transfer by this route of inoculation are pre-
Uterine Adenomata in the Rabbit. II

sent in Table II. Growth occurred in only 41.05 per cent of the animals used and the success of inoculation was extremely irregular in the different serial generations. 100 per cent of takes was obtained at one transfer while other transfers resulted in complete failure. The more successful transfers were made during the fall and winter months.

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<td></td>
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<td></td>
<td></td>
<td></td>
<td>95</td>
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</tr>
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and the largest per cent of takes resulted from inoculations made in February and early March. On the other hand, the failures occurred in the late spring and summer.

A distinction between the effects of adaptation and of season on the behavior of the testicular transplants is extremely difficult in the present series. Extreme seasonal variations are known to occur and
the present series of experiments were undertaken at different seasons. Thus, while the success of the first generation transfers appears to be directly related to the length of time that the tissue used had previously been serially transplanted in the eye, there is no indication in the data as to whether season or adaptation to transplantation was the determining influence. It is evident, however, that the percentage of takes in subsequent testicular generations was not increased by continued passage in that organ.

The percentage of takes was not consistently increased by shortening the interval between transfers but here again the influence of season may have been operative. On the other hand, the latent periods in the first generation transfers averaged 30 days irrespective of the season in which inoculations were made. Moreover, this period decreased to 14 days in subsequent generations and was not altered with changes of season.

The rate of growth varied within wide extremes but the variations were more marked between animals of different genetic constitution in the same generation than between similar animals in different generations. In some animals, the growing nodule never progressed beyond the size of a pea and was morphologically distinguished by an intense connective tissue proliferation and an acinar arrangement of epithelial cells (Fig. 5). On the other hand, the majority of animals developed multiple nodules which grew rapidly and completely replaced the testicle by the 40th day after inoculation. Histologically, such tumors resembled the rapidly growing transplants in the anterior chamber and were characterized by large confluent cellular masses with centrally placed areas of necrosis (Fig. 6). Growth was both expansive and extensive in character, destroying testicular parenchyma both by pressure and by active infiltration.

Infiltrative growth was limited to the testicular parenchyma for a long period of time and extension to the tunica vaginalis or to the spermatic cord was not observed before the 160th day. Expansive growth continued and eventually with encroachment on the blood supply, fluctuating necrotic areas appeared. Occasionally the entire testicle was converted into a sac distended with black fluid necrotic material, but even in such instances active nodules of growth were found on histological examination. In the majority of cases, however, such degenerative changes were limited to small areas and connective tissue replacement rather than necrosis characterized the older growths. The connective tissue was, in turn, invaded by tumor cells growing in strands and in isolated acinar groups. Growths of this type reached a large size and after the 160th day the testicle frequently measured 7 × 5 cm. and was characterized clinically by a firm nodularity. The eventual outcome of such cases is not known. One animal killed
on the 216th day showed fibrosis of the testicle with a complete destruction of all tumor cells, while the growth in another animal held for 240 days is still increasing in size.

Metastases have been found in three instances. The lymph nodes of the mesentery of the large intestine were involved in one animal killed on the 90th day and in a second animal killed on the 113th day which, in addition, showed a large metastatic nodule in the substance of the diaphragm. The third animal was killed on the 233rd day and secondary growths were found in nearly all organs of the body (Fig. 7). Microscopically the structure of the secondary growths was similar to that of metastases arising from transplants in the anterior chamber.

**Simultaneous Transfers to Different Sites**

Bilateral growths have been obtained from the simultaneous inoculation of both testicles in the same frequency with which inoculations into a single testicle have proved successful. Simultaneous transfers to the testicle and to the eye have resulted in growth in the eye alone in three instances and in growth in both locations in two instances. It is of interest that in one of the latter cases a metastatic growth was found at autopsy in a lymph node of the anterior triangle of the neck, while metastases were not found in the drainage area of the testicle.

**Reinoculation of Refractory Animals**

Transplantation of the tumor into the testicles following a primary unsuccessful inoculation has been attempted in 13 animals. The same testicle was used in the second attempt throughout the experiments. Control inoculations were made and gave a high percentage of takes. Reinoculation of 12 of the animals was performed 35 days after the primary failure and was unsuccessful in all instances. On the other hand, reinoculation was delayed for 69 days in one animal and resulted in a take.

**Transplantation into the Anterior Chamber of Animals with Spontaneous Uterine Tumors**

The transfer of tumor fragments into the anterior chambers of 8 animals bearing spontaneous tumors of the same nature resulted in 5 takes, an incidence of about 60 per cent in contrast to an incidence of
The transfers were made coincidently with the 1st, 2nd, 4th, 5th and 9th serial transplantations previously described and tumor material of the same derivation was transferred to both types of animals, but the incidence of takes in tumor bearing animals was always less than in normal animals of the same generation and did not increase with the incidence of takes in normal animals.

The latent period was of similar duration in both types of animals and the subsequent progress of growth in three of the tumor bearing animals was comparable with that observed in normal members of the same generation. One animal was killed on the 60th day and the tumor which occupied approximately 2/3 of the chamber showed the usual histological characteristics noted at that period. In another instance, the growth had replaced the anterior chamber on the 83rd day and had invaded the posterior chamber when the animal was killed on the 141st day. The anterior chamber of the third animal was completely filled on the 52nd day but at autopsy on the 105th day the tumor had almost entirely disappeared and the remaining portion was largely necrotic.

The progress of growth in the remaining two animals differed radically from that noted in normal animals. The growth rate was extremely slow in both instances and the transplants had no more than doubled in size after 130 days. At this time the animals were killed. Microscopic examination showed that the growth in one animal was almost entirely necrotic while the tumor in the other animal was characterized by an abundant myxoid stroma with epithelial cells arranged in well defined acinar formations (Fig. 8), despite the fact that the tumor in normal animals of the same generation showed an almost solid cellular structure with a minimum of supporting elements.

The success of transplantation in these instances appeared to be directly proportional to the size and age of the spontaneous tumors as judged by morphological examination and by a study of the breeding histories of the affected animals. The transfer of fragments to animals with small, early tumors resulted in three failures and two small slow growing nodules, while the inoculation of animals with large, older tumors was followed by takes and rapid growth in all cases.

**Other Reinoculation Experiments**

A series of experiments was designed with the view of obtaining more information regarding the effect of a growing tumor on the transplantation of other tumors of the same and of diverse nature. The performance of the experiments has been delayed because of the low
incidence of takes in the testicle during the summer months, which forced a temporary discontinuance of the series. While relatively few of the experiments have been completed and the available data do not justify conclusions, the results so far obtained seem to be of sufficient interest to warrant recording.

The inoculation of the left testicle with tumor material obtained from the right testicle after its removal from the body has been attempted in two instances. In one, the right testicle was removed 35 days after a successful transfer and the immediate inoculation of the left testicle resulted in a take. The subsequent growth of the nodule in the left testicle was similar in all respects to that previously observed in the right. In the second instance, on the other hand, the affected testicle was not removed until the 135th day and inoculation of the remaining testicle was not followed by growth. Control inoculations resulted in 57.1 per cent of takes in the first instance and in 100 per cent of takes in the second instance.

An attempt to inoculate the testicle with material obtained from biopsy of the eye 66 days after transplantation into that organ was likewise unsuccessful although the inoculated tissue grew in the testicles of all of the control animals.

In another experiment, the tumor was successfully transplanted to the anterior chamber of the right eyes of 13 young animals. The chambers were eventually filled by the growth which subsequently underwent complete regression and left extensive corneal scars. 167 days after the first transplantation, fragments of an actively growing tumor derived from the eye of a member of the 11th serial generation were transferred to the anterior chamber of the left eyes of the recovered animals and 8 controls were inoculated at the same time. Growth resulted in all of the controls but in no instance did a take occur in the reinoculated animals.

Postmortem Examination

A detailed postmortem examination was made of all animals included in these experiments, and in view of the changes found in animals bearing spontaneous uterine tumors, particular attention was directed toward the endocrine system. In the majority of cases, the organs were not pathologically altered and lesions that were found
were traceable to disorders common in the general animal population. In no instance were changes comparable to those observed in animals bearing spontaneous uterine tumors found in animals bearing transplanted uterine tumors.

Inoculation with Dried Frozen Tumor Material

A watery emulsion of dried frozen tumor material was inoculated, at laparotomy, into the uterine mucosa of 3 virgin females and 3 multiparae. The animals were killed 42 days after inoculation and the uterine mucosa was serially sectioned. Microscopic examination of these sections and of sections from other organs showed no alteration from normal. Controls inoculated with the living tumor, on the other hand, gave 100 per cent of takes.

DISCUSSION

In the report dealing with spontaneous tumors of the uterus, especial emphasis was placed upon the constant occurrence of certain endocrine changes most evident in the pituitary, suprarenals and thyroid. In brief, the alterations in the pituitary were productive in nature while those in the thyroid and suprarenals were retrogressive. These alterations were present from the earliest stages of tumor development and were also found, before histological evidence of neoplasia, in older animals of the tumor line. This, in itself, suggests the possibility of an antecedent change in the endocrine mechanism which had some bearing on the eventual development of the neoplastic process. This suggested relation is supported by the fact that the conditions observed in animals with spontaneous tumors bear a striking resemblance to those in animals subjected to long continued treatment with estrone, and from this it was inferred that spontaneous tumor development might represent a natural analogue to the experimental production of neoplasia with such substances.

The point to be emphasized in the present discussion is that, despite the fact that the uterine tumor under consideration has been successfully transplanted into more than 150 rabbits and has grown progressively for periods up to 11 months, none of these animals has shown changes at autopsy which bore the slightest resemblance to those constantly present in animals with spontaneous tumors. It is
apparent, therefore, that the endocrinological changes are not produced by the continued growth of neoplastic cells. It is also clear that the constitutional status associated with the endocrine changes is not an essential factor in the continued growth of neoplastic cells. In fact, the failure of transplanted tumors to grow actively or to grow at all in animals with early spontaneous tumors suggests that initially there is a contrary or inhibitory influence associated with such changes. It would appear then that the endocrinological conditions referred to were concerned with the initiation of neoplasia and that in the study of this tumor there are, as usual, two distinct problems to be considered, namely: the initiation of neoplasia and the continued multiplication and growth of neoplastic cells.

Investigation of the first problem is being carried out from the point of view of an exogenous as well as of an endogenous origin. Experiments based on the suggestion that the endocrine changes were associated with the initiation of neoplasia have been most encouraging and will be reported at a later date. On the other hand, attempts to demonstrate a causative agent of the nature of a filterable virus have so far been unsuccessful. Neoplasia was not initiated by the introduction of dried frozen material into the uterine mucosa. It is obvious, however, that numerous experiments of this character are necessary before a conclusion is warranted and such experiments are being continued.

The growth characteristics of the tumor have been studied in the eye and in the testicle. Both of these sites have been used by other workers in the investigation of other tumors. The testicle came into prominence following the work of Brown and Pearce with the transplantable epithelioma. They also used the anterior chamber in early experiments and reported a uniform series of takes with rapid growth to large tumors which, however, did not metastasize during the period of observation. A single case of spontaneous regression was noted (4). A sarcoma of the lower jaw had previously been successfully transferred to the anterior chamber by Schultze (5) and Happe (6). Their general results were in agreement with those of the present study but a further investigation of the characteristics and potentialities of the chamber as an inoculation site does not appear to have been carried out. The anterior chamber has also been successfully used in homol-
ogous transplantation experiments with tumors of other species (7) and was employed in many early heterologous transplantation attempts without apparent success.

While the susceptibility of the anterior chamber to tumor transplantation appears to be well recognized, it has not been widely used as an inoculation site. Certain features associated with its susceptibility and the characteristics of the resulting growth suggest, however, that a more general use might be of advantage. A comparison of the frequency of takes of the uterine tumor in the testicle and in the eye, as in Table III, brings out a number of points of interest. The relatively unimportant reduction in the number of takes in the anterior chamber during the summer months as compared with the complete failure of testicular inoculation suggests that this method of transfer might prove of great value in the maintenance of other tumors which are so frequently lost in testicular and subcutaneous series during this season.

In the series as a whole, the number of takes resulting from transfer to the anterior chamber was twice as great as followed inoculation into the testicle. This finding is in line with the fact that in no instance were first generation transfers from a spontaneous tumor to the testicle successful and that takes in the testicle were not obtained until the tumor had been passed through three generations of animals by serial eye transfer. While the cellular damage incident to the preparation of an emulsion may have been of influence in the lower frequency of takes in the testicle, the fact that 100 per cent of takes occurred at some transfers without modification of the technique indicates that this was not an important factor. It seems more prob-

| TABLE III |
| A Comparison of the Percentage of Takes Resulting from Transfer to the Anterior Chamber and to the Testicle throughout the Year |
|------|------|------|------|-----|------|------|------|-------|------|------|
| Anterior chamber | 100  | 83.3 | 55.5 | 100 | 76.9 | 100 | 75   | 33.3  | 100  | 87.5 | 87.5 |
| Testicle        | 25   | 62.5 | 64.7 | 42.8 | 0    | 37.5 | 25   | 0     | 50.0 | 66.6 |
able that the greater success in the anterior chamber is related to the slower reaction of the tissues of the eye to the presence of the transplanted fragment. The fragment grows for a longer period of time in the manner of a tissue culture and a degree of adaptation results before the occurrence of a foreign body reaction with the intimate contact between the cells of the host and the graft that determines its immediate fate.

Transplantation into the anterior chamber offers the further advantage of continuous visual examination of the graft. The effects of various procedures can be watched and the rate of growth can be measured easily with a pair of calipers. Metastasis may occur earlier and with greater frequency from testicular growths but the continued life of the animal is an asset in certain types of experiment. On the other hand, there are distinct disadvantages associated with ocular transfers. The anterior chamber is a small confined space surrounded by relatively inelastic tissues and the increased pressure which follows rapid growth may lead to necrosis and regression of the tumor. If the cornea is invaded or ruptured, the external extension of the tumor produces an unsightly fungating mass and trauma may lead to severe hemorrhage or infection. The great advantage of this method of transplantation lies in the relative ease with which first generation transfers from a spontaneous tumor can be effected. During the past four years more than 140 spontaneous rabbit tumors representative of 16 different types of growth have been observed in this laboratory. Attempts were made to transplant the different types of growth and until recently the testicular and subcutaneous routes were almost exclusively employed. It is significant that while these methods of inoculation failed in every instance, four out of five attempts to transfer by means of the anterior chamber proved successful.

It is not known whether the lowered incidence of takes during the summer months, particularly in the testicle, is related to an increased resistance of the animals or a decreased activity of the tumor cells. There is evidence, however, that even in winter months, periods occur during which an animal may be refractory to inoculation and it is conceivable that meteorological conditions more prevalent during the summer may bring about a refractory phase of widespread occurrence.
The spontaneous and the transplanted tumors show many comparable characteristics. In both abnormal and normal hosts, the tumors progress slowly, growth is at first expansive and later infiltrative, and metastasis is a late occurrence. One characteristic of especial interest is shared by the transplanted tumor and by metastases of the spontaneous tumor. In both, there is evidence of an ability of the neoplastic cells to respond to different environmental conditions with an alteration in the degree of differentiation. Thus, in certain situations, metastatic cells grow in a well formed acinar arrangement and appear to be further differentiated than the cells of the primary growth. In like manner, the transplantation of a cellular, poorly differentiated tumor into animals of a special genetic constitution is followed by more highly differentiated growth with the formation of more or less typical structures.

In other respects the transplanted tumor behaves in a different manner and it should be emphasized that the behavior of neoplastic cells in normal animals is not a reflection of their behavior in the primary host where their activity may be influenced by an altered endocrinological status as well as by the presence of other growing neoplastic cells.

The observation has been made repeatedly by workers with some other tumors that, following successful transplantation, a phase occurs during which the animals are refractory to further inoculation of the same growth (8). In the present instance, it was also found that reinoculation gave negative results after continued growth of the transplanted tumor. Further conclusions cannot be drawn from the results obtained to date, but certain findings in regard to the refractory phase are of interest from the point of view of discussion.

Despite opinions to the contrary, it appears to be fairly clear that the refractory phase is brought about by the continued presence and growth of neoplastic cells, rather than by the absorption of products resulting from regressive changes in the tumor. The duration of the refractory phase in experimental animals in which the tumor under discussion had completely regressed was demonstrated to exceed 5 months. On the other hand, metastasis has occurred in other animals 3 months after transplantation. There is some evidence, there-
fore, that while growing neoplastic cells may bring about a refractory period, their continued presence and growth in the body may shorten the duration of this period.

The presence of a similar refractory phase in animals with spontaneous growths has not been satisfactorily demonstrated. It is generally believed that grafts of a spontaneous tumor are more apt to be successful if placed in another region of the same animal than if transferred to a normal animal and, in early experiments with the present tumor, it was found that subcutaneous autografts almost invariably grew while subcutaneous transfers to normal animals were uniformly unsuccessful. In these experiments, the growth had been present in the spontaneous host for more than a year before auto-inoculation was attempted and it is apparent that at this period of tumor development the animals were not refractory.

A determination of the susceptibility of animals in earlier stages of tumor growth to the transplantation of a malignant tumor of the same nature is a more difficult problem owing to the necessity of assembling a sufficient number of suitable animals as well as of determining the age of the spontaneous tumors. It should also be emphasized that in this type of experiment, in contrast to the experiments cited above, autogenous tumor material cannot be used inasmuch as in the early stages it represents benign rather than malignant neoplasia.

The occurrence of a considerable number of spontaneous uterine growths, the age of which could be determined with fair accuracy, offered a unique opportunity for study of this problem, and tumor material derived from various eye generations was transferred to the eyes of animals bearing spontaneous growths in different stages of development. A series large enough to provide significant results has not been tested and the experiment is being continued as more tumor bearing animals become available. The results are not conclusive for this reason but, while it must be borne in mind that further tests may give rise to contradictory findings, the present trend is of sufficient interest to warrant some consideration.

The uniformity of takes in animals with old tumors is in agreement with expectations based on experiments with autografts. On the other hand, the complete failures and the small slow growing nodules arising from transfers to animals with early spontaneous tumors are
more in line with the results obtained from the reinoculation of animals bearing transplanted tumors and may indicate the existence of a similar refractory phase. Additional evidence suggesting the presence of a growth inhibiting influence at this period is given by the fact that while neoplastic cells are present in the blood stream at early stages of tumor development, they fail to gain a foothold and grow until late in its course.

SUMMARY

The behavior of a transplanted adenocarcinoma of the uterus of a rabbit has been studied through 12 serial generations in the anterior chamber of the eye and 6 serial generations in the testicle. The transplanted tumor is characterized by slow growth which is at first expansive and later invasive, by an ability to form more or less differentiated structures in response to different environmental conditions and by late metastasis. The endocrinological changes that distinguish animals bearing the spontaneous tumor do not occur in animals bearing the transplanted tumor.

Various experiments were undertaken in an attempt to discover the nature of the factors determining the characteristics of the spontaneous and of the transplanted tumor. It was found that successful transplantation was followed by a phase during which animals were refractory to reinoculation. The results of transplantation into the eyes of animals with spontaneous tumors suggested the existence of a similar phase during the early development of the tumor but the number of observations was not sufficiently numerous to warrant definite conclusions.

BIBLIOGRAPHY

EXPLANATION OF PLATES

Hematoxylin and eosin was the stain employed throughout.

PLATE 28

Fig. 1. Section of a transplant in the anterior chamber of the eye resulting from the 2nd serial transfer. Stroma is abundant and epithelial elements tend to grow in abortive acinar formations. × 67.

Fig. 2. Section of a transplant in the anterior chamber resulting from the 6th serial eye transfer. In contrast to the previous figure, stroma is sparse and epithelial cells are arranged in large rounded masses which frequently show necrotic centers. × 67.

Fig. 3. Section of a metastatic growth in the ovary derived from a transplant in the anterior chamber. × 67.

Fig. 4. Section of a metastatic growth in the pancreas derived from a transplant in the anterior chamber. × 40.5.
Photographed by J. A. Carlile

(Greene: Uterine adenomata in the rabbit. II)
PLATE 29

FIG. 5. Section of a transplanted tumor in the testicle of a Himalayan rabbit. Stroma is abundant and epithelial cells grow in acinar-like groups. × 67.

FIG. 6. Section of a transplant in the testicle of a hybrid rabbit showing the characteristic histological features found in the majority of testicular grafts. In contrast to the appearance of the tumor in Himalayan animals, stroma is scanty and the arrangement of epithelial cells is similar to that found in rapidly growing eye transplants. × 67.

FIG. 7. Section of a metastatic growth in the lung derived from a testicular transplant. × 67.

FIG. 8. Section of a transplant in the eye of a rabbit bearing an early spontaneous uterine tumor of the same nature. Stroma is abundant and epithelial cells are arranged in well defined acinar formations. The appearance of control transplants in normal animals is shown in Fig. 2. × 375.