INTRAOCULAR HETEROLOGOUS TRANSPLANTATION OF AN AVIAN LYMPHOID TUMOR

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INTRAOCULAR HETEROLOGOUS TRANSPLANTATION OF AN AVIAN LYMPHOID TUMOR

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INTRODUCTION

Since the advent of the present century considerable research has been directed toward finding suitable media for growing cells and tissues in a controlled manner. Great strides have been made by the use of chicken embryo inoculation; likewise many contributions gained from tissue culture techniques. During the past dozen years, although it is by no means a new method, the transplantation of tissue into the anterior chamber of the eye has been exploited as such a biological tool.

The import of transplantation of tissues to the anterior chamber of the eye was recognized by Loeb[1] (1945) in substantiation of his "individuality differential". This is defined as the particular characteristic common to all the various tissues and organs of an individual which distinguishes one individual from another. According to this investigator, there is inherent in every higher individual organism something which differentiates it from every other organism. This can be discovered by observing the reactions of certain cells and tissues belonging to one individual

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[1] Leo Loeb, late Professor of Pathology, Washington University and author of The Biological Basis of Individuality from which much of the significant basis for this thesis was derived.

towards the cells and tissues of another individual of the same species.

In the same manner, there are characteristics common to all members of a species, genus, order and class, which may be called species-genus-order-class differential. Significance should be attached to the class differential with which we are concerned in the present experimental work. Superimposed upon this fact is the tissue differential of the lymphoid tumor which differentiates it from any other tissue of the avian donor.

The anterior chamber possesses, apparently, the unique distinction of being that part of an individual in which the least immune response is evidenced. Generally, transplanted tissues, though varying in ability to survive, can grow to a greater extent in the anterior chamber of the eye than at any other site. Less highly differentiated tissues respond more readily to transplantation than their more highly specialized counterparts. Much use of these facts has been made in transplanting tissues, both normal and neoplastic, into the eye of a host animal from another part of the same individual, or from another member of the same species or related species, or even from those not distantly related. Varying degrees of relationships, to some extent, can be determined by observing the reaction provoked in the anterior chamber of the eye of a host by the tissue of another individual.

Transplantations are categorized in accordance with the relationship of the host to the donor of the tissue transplanted. If tissue is relocated from one part of the body to another of the same individual the process is known as autotransplantation.[1] Depending upon the phylogenetic relationship of individuals transplantations have been further classified by Loeb as syngenesio-, homo-, or hetero-transplantations. The first refers to transfer of tissues between siblings. Homotransplantation is a more general term inferring the transfer of tissues from one member to another of the same species. From the genetic standpoint, it can be seen that varying degrees of relationship could be established in a single species. Interspecific transfers are designated as heterotransplants.

The relationship between a piece of living tissue and the recipient may be such that sites other than the anterior chamber of the eye may be employed for transplantation. Such a relationship has facilitated bone grafting, corneal transplantation and certain cosmetic surgery techniques.

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[1]Present day investigators are not in agreement concerning the nomenclature of transplantation. The majority add the suffix "-ologous" to the word stem denoting the relationship of the host to the transplant, as for example, homologous. Loeb has appropriated the ending "-ogenous", as shown by heterogenous. Also used interchangeably with the above designations is the addition, as used in the text of this paper, of the word stem as a prefix to transplantation as autotransplantation. It is this investigator's opinion that any appropriate terminology may be employed correctly.

The views concerning growth of malignant tumors in heterologous species are not wholly confluent. Recently the hypothesis has been advanced that malignancy is characterized by autonomy. According to Greene[1], (a) normal adult tissue, (b) inflammatory lesions, and (c) benign tumors fail to survive while (d) embryonic tissues and (e) malignant neoplasms grow after intraocular transplantation in heterologous species. The growth of a tumor in the anterior chamber of the eye of a heterologous species would confirm the diagnosis of malignancy. Thus malignancy, according to this investigator, approaches the threshold of immortality. This belief has not found immediate acceptance among investigators. The disagreement lies not with the growth of a particular neoplasm intraocularly in an alien species, but rather whether or not this growth confers a designation of malignancy upon the original tumor. Likewise, does a growth that fails to be transplanted heterologously signify benignancy?

Although the inciting cell-free agent of this tumor was apparently present at all times, this study was concerned only with the neoplastic cells. Several interrelated questions were posed as the initial studies were made.

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[1]H. S. N. Greene, the leading investigator of the potentialities of the anterior chamber of the eye as a research diagnostic tool. Department of Pathology, Yale University School of Medicine

Foremost of these concerned whether or not a successful heterologous transplantation could be made between such widely biologically separate species as the chicken and the guinea pig with a lymphoid tumor. Significance was also placed upon the degree of reaction provoked in the host by the transplanted tumor. The possibility of reestablishing the tumor in its original host to study any altered pathology was also considered. Specificities as the rate of growth, maximum size, optimum growth period for harvest and serial passage were included as essential data to be gathered from this study. In an endeavor to gain a greater understanding of the properties of an avian lymphoid tumor maintained at the Regional Poultry Research Laboratory this problem was undertaken.

REVIEW OF LITERATURE

The suitability of the anterior ocular chamber for the transplantation of tissue was first demonstrated by Van Dooremaal (1873). Since that time excellent reviews of literature on this subject have been published by Lucké and Schlumberger in 1939 and Morris et al in 1950. Both reviews recognized that the history of utilization of intraocular transplantation by various investigators falls naturally into three periods.

The first intraocular heterologous transplantation attempts of a tumor by Zahn (1884) initiated an era that extended to 1912. A human hyaline chondroma failed to survive in the anterior chamber of the eye of the rabbit. At eighty days, when the animals were sacrificed, nearly complete resorption of the tumor had occurred. This work was further substantiated by Herzog (1902) who reported that he had tried for years, without success, to accomplish heterologous intraocular transplantation of carcinomas and sarcomas of human beings.

Later, Ruben (1912) was successful in making a homologous transplantation of Jensen's rat sarcoma, but heterotransplants into rabbits failed. These reports exemplify the apparent agreement among investigators of this period concerning the nontransplantibility of tumors from one species to another.

Reports of successful heterologous transplantations into the anterior chamber were made by Hegner (1913) and his associate Keysser (1913). In several cases they obtained growth of a human sarcoma transplant in rats demonstrating that the eye was a suitable habitat for heterologous tumors. Carcinomatous transplants failed to take and retrogressed.

Woglom (1915) was unable to substantiate the claims of Hegner and Keysser. Although a suspension of the Crocker mouse sarcoma 180 produced 45 takes among 54 control mice, it failed to grow intraocularly in rats. This second era was marked by disagreement among investigators concerning the validity of heterologous intraocular transplants.

The third period was ushered in by a report of Smirnova (1937) describing successful implantation into the anterior chamber of heterologous species transplants of a human mammary carcinoma and Ehrlich's mouse carcinoma.

By far the most intensive study involving heterologous intraocular transplantation to date has been made by Greene. Homologous transfers of an uterine adenocarcinoma of rabbits prompted preliminary investigations with Saxton (1938) in transplanting this tumor into guinea pig eyes. Progressive growth of the transplant was obtained using tumor material derived from the sixth serial eye transfer in the rabbit.

Lucké and Schlumberger (1939) initiated transplantation experiments by utilizing a naturally occurring renal carcinoma of the leopard frog (Rana pipiens). The tumor was introduced intraocularly into other members of the same species. Operations and photography of the transplanted tumors were performed under vaporized ether anesthesia. Over 50% of the transplants from ten of the tumors grew readily. They could not explain why transplants from three other tumors made little progress and were soon absorbed or become fibrosed.

An excellent description of the manner of growth as observed by slit-lamp microscopy was given by them. No other method affords such continuous observations of a living tumor in the anterior chamber. The pale color of the transplant contrasted sharply against the dark background of the iris. Within two to three days the ragged tags of tissue on the 1-2 mm. diameter piece of tumor were absorbed and the surface became entirely regular in outline. This condition persisted for several days, depending upon the temperature of the environment and the growth energy of the particular strain of tumor. During this period of lag no noticeable exudative reaction to the transplant occurred if infection was avoided.

Furthermore the manner of growth as dependent upon the contiguous structures of the anterior chamber was also included. The transplant generally became lodged upon the

iris, lens or less frequently the cornea. Although the attachment was limited at first, displacement didn't seem to hinder further development. The transplants which became fixed to the pupillary margin of the iris generally showed much better growth than those which lodged near the periphery. The reason given for this was the greater depth of the anterior chamber in the pupillary region affording increased gaseous and nutrient exchange between tumor and surrounding aqueous humor.

Observations led to the conclusion that the form which the growing tumor assumed depended on its immediate physical environment. Where the tumor grew out in the midst of the aqueous humor, unimpeded by solid tissue, its habit of growth was tubular or papillary. If, however, the tumor grew in contact with a firm even surface such as a lens or cornea, then the form of growth was entirely different; broad membranes were formed which extended and covered the surface. A third form of growth was recognized when tumors came in contact with loose discernible tissue, such as the iris. The tumor extended downward forming invasive tubules and alveoli within the prexisting stroma of the invaded tissue. The first two forms could be followed by slit-lamp microscopy whereas for the third type histological sections were required.

The interior of the original graft usually became more or less fibrous, while the cells at the periphery proliferated.

Occasionally backward extension into the posterior chamber, lens and vitreous took place; no extraocular extension was observed. Once the size of the tumor exceeded its requirement, gradual decline in size, regression, and increasing fibrosis appeared to be the general rule. The relatively small and confined space in the anterior chamber and the progressive increase in intraocular pressure with resulting circulatory disturbances seemed to be the important limiting factors.

After preliminary heterotransplantation of the rabbit adenocarcinoma, H-31, Greene (1941a, 1941b) expanded his study to include other rabbit and human neoplasms. Experimental transplants were continued in the guinea pig; other hosts were also employed. Transfers of H-31 were discontinued after the fifth serial passage in the guinea pigs as there was no cause to believe that the tumors could not have been perpetuated indefinitely in this manner.

Control inoculations of whole tumor fragments or of cellular emulsions into the testicles, muscles, and subcutaneous tissues of guinea pigs were performed throughout the series of experiments. Tumor tissue derived from guinea pig generations as well as from rabbit hosts was used, but no takes occurred.

Generally, the presence or absence of takes in the guinea pig's eye could be detected during the second week after transfer, and at this period a slight increase in size and a pinkish coloration distinguished growing fragments. Occassionally,

the transplants remained unchanged in appearance for as long as 160 days, but eventually grew to fill the chamber. Vascularization was usually apparent by the third week, but in instances such as the above, the fragments received no visible blood supply during the latent period.

Growth proceeded at a slower rate than in the rabbit and 40 to 50 days were generally required before such a size was attained. Thereafter areas of degeneration appeared and the tumors regressed. On the contrary, small nodules of healthy tissue presisted and by continued growth again filled the chamber.

At necropsy the tumors appeared as semi-translucent, pinkish masses. The greater part of the iris was often replaced by the tumor tissue of older transplants, but the cornea was never invaded and the growth never extended into the posterior chamber.

Microscopically, the tumors resembled those resulting from homologous transfers. The epithelial elements were identical in appearance and manifested the same tendency to grow in small acinar bundles or in large, solid, cellular masses. The stroma was abundant, well vascularized, and healthy in appearance. Nowhere were there lymphocytic infiltrations or evidence of a foreign body reaction. Serial sections were obtained from many organs, but in no instance was a lesion found which could be unequivocally identified as a metastasis.

This tumor was successfully transferred to the eyes of swine and goats. Intratesticular inoculation of a cellular emulsion failed to result in takes in swine.

Successful heterologous transplantation of the tumor T-36, was effected in guinea pigs, swine and sheep. The original tumor tissue was obtained from a splenic metastasis of an acinar type breast carcinoma which had been carried by serial anterior chamber transfer through more than 50 generations of rabbits.

Both young and old guinea pigs were used in the serial transplantation experiments. The percentages of takes and course of the tumors were similar in both cases, but the growth rate was slower in the older animals and takes could rarely be recognized before the tenth day. On the other hand, growth was almost invariably evident by the fifth day in young guinea pigs, and subsequent progress was rapid. Occasionally, growth ceased and regression occurred before the chamber filled, and the animals of this type were found to be refractory to reinoculation. In the majority of cases, however, growth continued until the chamber was filled. This usually occurred by the tenth day in young animals, while in older guinea pigs two to three weeks were required. Further increase in size was attended by bulging of the cornea and degenerative changes in the tumor, but in general, the tissue remained viable and could be transplanted for as long as a week after filling the chamber. Occasionally the

cornea ruptured in consequence of the increased pressure and the tumor protruded externally. Infection and spontaneous regression invariably occurred in such cases.

Growth was progressive and continued throught the life of the guinea pig in 5% of the cases. This was a special feature of the guinea pig and was not observed in the rabbit. It occurred almost exclusively in guinea pigs inoculated in February and March, and apparently bore no relationship to the status of the animal or the rate of growth. In such cases, external extension was a gradual process and occurred after a period during which the intraocular growth assumed the shape of a cone and the area widened to form a carcinomatous ulcer. Eventually the cornea was destroyed and the tumor protruded as a fungating mass. At necropsy the tumors were found to have extended laterally under the conjunctiva and aposteriorly into the vitreous humor. The retina and choroid were often destroyed but the tumor did not invade the sclera. The regional lymph nodes were enlarged but metastases were not visible throughout the body.

At intervals throughout the experiments tumor tissues from guinea pigs was transplanted back into rabbits. Takes occurred in all instances and the characteristics of the resulting tumors were in no way different from those observed in serial rabbit transfers.

The papillary type rabbit breast carcinoma, B-240, was successfully transplanted to the eyes of guinea pigs, but

transfer to other alien species was not attempted. Six attempts to transfer the Brown-Pearce rabbit tumor to guinea pigs terminated in failure.

Prior to this work, it was observed that endometrium or breast tissue obtained from normal adult rabbits as well as embryonic parts containing mixed tissues could be grown indefinitely in the anterior chamber of the eyes of other rabbits. Adult tissue was found to increase in size at a very slow rate, and no attempt was made to carry the tissue for more than a single generation. On the other hand, embryonic tissue grew rapidly, and in one experiment fragments derived from ten day old fetuses were carried for six serial generations.

Fragments of normal adult rabbit endometrium were transplanted to eyes of nine guinea pigs. On the seventh day the transplants of eight of the animals were pinkish in color and apparently living, while in one animal these fragments were opaque and white. The fragments were still living on the nineteenth day and histological examination showed the presence of a vascular supply and the absence of any degenerative process or foreign body reaction. However, by the twenty-fifth day, the pinkish coloration had disappeared and portions of the transplants were opaque and white. The animals were killed on the twenty-eighth day, and on microscopic examination the fragments were found to be in an advanced stage of degeneration.

Almost identical results occurred with the normal breast tissue transplants. The embryonic tissue, on the other hand, survived and increased in size until the animals were killed at the end of a month. The rate of growth in guinea pigs however, was very much slower than in the rabbit and the fragments had only doubled in diameter when the experiments were terminated. Moreover, histological examination showed that survival and growth were limited to skin and cartilage and that all other tissues of the original graft disappeared.

More thorough reviews of the work of Lucké and Schlumberger and Greene were deemed advisable to present a clearer understanding of the potentialities of the anterior chamber. Various stages of interaction between the transplant and the host were observed and initial criteria of successful transplantation were given. Much of the experimental basis for this thesis was derived from material presented by these investigators.

The second publication by Greene (1941b) concerned the transfer of human tumors to alien species, paralleling the preceeding work with rabbit neoplasms. Additional criteria were formulated to lend understanding to the manner of growth of intraocular transplants.

In all tumor transfers it was clear that the transplanted cells lived for a period of time in the manner of tissue culture; tumor cells subsisted on materials introduced with the

inoculum, but continued survival was dependent upon the imbibition of nutrients from the surrounding tissue fluids.

Greene related that the iris was relatively unresponsive to the presence of foreign bodies and that the interaction was delayed for a considerable period of time. During the interim, the transplanted fragment persisted as a free entity deriving nourishment from the fluid of the chamber and its cells remained entirely independent of the tissue of the host. Growth proceeded during this culture-like phase and an increase in size was evident before the occurrence of vascularization. An inflammatory reaction did not occur. On the contrary, the transplant, which at first survived as a parasitic tissue culture, became supplied after a time with a rich plexus of blood vessels, was incorporated in the body of the host, and entered into intimate relationship with the organism, as if transplanted to an animal of the same species.

Greene further stated that a foreign body reaction was not immediate but occurred after a variable interval and in variable intensity depending on the donstitution of the transplant and host and on the sensitivity of the region used as an implantation site. In ordinary homologous transfers, the foreign body reaction was inconspicuous and consisted largely of a proliferation of fixed tissue elements which eventually resulted in vascularization of the fragments.

On the contrary, the transfer of foreign tissue invoked an intense reaction, acting as an inflammatory stimulus. Free mobile elements of the blood and lymph migrated to the tissue resulting in an eventual encapsulation.

If the foreign body reaction was delayed and the transplant lived, it was conceivable that its cells were gradually altered by the continued imbibition of host materials so that some degree of adaptation resulted and the more radical constitutional differences between transplant and host were lost. The eventual foreign body reaction was less intense and more in the nature of that which followed homologous transplantation.

Invasion of normal tissues occurred but usually expansive growth proceeded at a greater rate and the increased pressure incident to the rapid filling of the chamber interfered with the blood supply and regression followed.

Greene found no existing criteria for a comparison of the behavior of human tumors in the eyes of alien species. However, the long period of quiescence which followed transfer to the new species was suggestive of the interval which frequently separated surgical growth and local recurrence in the human host. Evidence of growth was based upon (1) directly observed growth of transplanted fragments, and (2) presence of invasion in microscopic sections.

Attempts to transplant the Brown Pearce tumor, a epidermoid carcinoma of the scrotal region of the rabbit to

the guinea pig failed. The conclusion was given that the growth was not transferable to this species by the methods employed.

The mouse eye was used by Greene (1945) in order to lessen the expense of securing and maintaining laboratory animals.

Data obtained with this study showed some interesting trends which facilitated the classification of species with respect to ease of heterotransplantibility. Transfers from man to mouse eyes grew better if they were first passed through a guinea pig generation. In contrast, the mouse was found to be a better host for rabbit tumors than was the guinea pig. Consequently, the mouse and the rabbit were placed in one group, and man and the guinea pig in another one in accordance with the ease of transfer within the group and the difficulty between groups. This also represented a division with reference to the ability to synthesize vitamin C. The former group possessed the ability and the latter group was not able to produce this vitamin in the intestinal tract. Greene felt that this was possibly coincidental but metabolic differences between tumor and host tissue presumably occurred and might possibly have accounted for the observed variations.

Greene and Murphy (1945) obtained nearly 100% takes by heterologous transfers of a bronchogenic carcinoma of mice and rats to the eyes of hens and ducks. Vascularization

of fragments was apparent after the second week of transfer, however, subsequent growth was extremely slow.

The property of heterotransplantibility was not shared by all tumors of these species but appeared to be a common characteristic of a special group, thus was offered a means of classification based on biological behavior. This special group was characterized by distinctive properties with respect to manner and range of growth in the parent species. In general, they were distinguished by the capacity to invade and metastasize and to grow in unrelated[1] animals of the same species. This ability characterized the heterotransplantable tumors of all the species studied. The same ability was apparently an essential property of neoplasms that were capable of survival and growth in unrelated strains of the parent species.

Greene hypothesized that the basis of classification of benignancy and cancer was justified by the distinction of autonomy of the latter grouping. Concerning the evolution of malignancy, it was suggested that at the early stages of oncological development, when only limited transfer was possible, the tumors were dependent for continued survival and growth on factors peculiar to the primary host.and closely

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[1]The phrase "unrelated animals of the same species" should be clarified at this point. For example, Greene found that all mouse tumors appear to be transplantable in animals of the strain in which they originate. This doesn't constitute homologous transfer, for as a result of long inbreeding the donor and recipient in such an experiment bear a genetic relationship somewhat comparable to that of the fore and hind quarters of the same individual.

related individuals. These factors, not being supplied by unrelated individuals, such animals would not support growth of tumors on transfer.

At a later stage autonomy was attained; the tumors became independent of factors concerned in this development, and gained the ability to survive and grow in their absence. The independence thus achieved eliminated genetic and species barriers and allowed growth in unrelated animals and in alien species.

Shrigley, Greene and Duran-Reynals (1945) reported a variation of the Rous sarcoma virus following growth of the tumor in the eye of the guinea pig. Criteria for growth achieved in ten to twelve days was based on (1) abundance of mitotic figures, (2) actual increase in size of the transplant, and (3) finally when the anterior chamber was irrigated with dilute cell suspension growth resulted from the widely dispersed sarcoma cells. It was interesting to note this report as the first in which the species of both the host and donor of the transplant were the same as the present investigation.

The behavior of morphologically comparable mammalian cancers in heterologous species differed from that of the Rous sarcoma in several ways. As a rule, the appearance of vascularization was delayed for a week or more and no increase in size was observed for several months. The tumors would then grow rapidly to fill the chamber. With

the conditions of this study, vascularization of the transplant occurred very soon after transfer, as early as three or four days. Growth was very rapid during the first two weeks, then quiescent and showed no change in size for as long as six months. During this time the tumor appeared alive, for microscopic examination disclosed no degenerative changes and occasional mitotic figures were formed.

Another point of dissimilarity was the difficulty with which the Rous sarcoma was passed serially in the guinea pig. In the case of mammalian tumors serial transfer was easily effected and apparently could have been continued indefinitely. Shrigley et al (1945) successfully passed the Rous sarcoma for only two consecutive transfers in the guinea pig. On the other hand, after a single passage in the chicken, return to the guinea pig was followed by growth.

Variation of Rous virus which followed growth of the tumor in the anterior chamber of the guinea pig eye were: (1) periosteal tumors developed, (2) hemorrhagic disease, a non-neoplastic manifestation of the free virus, occurred in tissues not frequently affected by a stock Rous virus, and (3) there was a suggestion of an alteration of species affinity for the chicken by one variant Rous strain.

It had been shown previously by Duran-Reynals (1942, 1943) that the Rous sarcoma virus was modified by inoculation of the tumor into ducks, guinea fowls and turkeys. The variation of three strains of Rous sarcoma virus after

passage through the guinea pig eye was more extreme than turkey and guinea fowl variants and less extreme than the duck variant.

The title of Greene's (1946) next paper, "The Microscope or the Guinea Pig" exemplified the ever increasing faith in the anterior chamber of the eye as a biological tool. The growth of human malignancy in the guinea pig eye was often associated with a higher degree of cellular differentiation and structural organization than was present in the parent tumor. Thus, the value of heterotransplants as an aid to the diagnosis and classification of human tumors was emphasized. Greene held to the theory that the mere fact of growth immediately identified the tissue in question as cancer. Nevertheless, conclusive demonstration was made of the potentialities of dynamic pathology as contrasted with the limitations of static morphology.

Dyer and Kelly (1946) in an endeavor to make available for study a regular supply of fresh tumor material from a selected cancer of human origin selected the procedures of Greene. Successful vascularization and growth of transplants were observed in the eyes of 27 of 66 guinea pigs of 17 different groups. Failure to obtain growth of transplants of seven of 14 clinically proved cancers, the transient character of the transplants that did grow, and

finally the small amounts of tumor material present at the time of maximum growth indicated that this method was not practical for routine growing of human tumor tissue.

The high degree of anaplasia and the early metastatic dissemination observed in many cancers of childhood were suggestive of considerable autonomy. Eichwald (1948) reported the results of the heterotransplants of 27 of these tumors of nine diagnostic types into 158 guinea pigs. Only one, a congenital fibroscarcoma, was a successful transplant.

Continued heterotransplants, as such, of the Brown Pearce tumor into guinea pig eyes failed in all attempts. Greene (1949), however, reported success with transplants into mice, rats and hamsters. One important difference in behavior of this tumor was noted. No preliminary passage into the eyes of the host was necessary as with other tumors before subsequent successful transfer could be made to different bodily regions of other individuals. Transplants of this neoplasm from a rabbit's eye was followed by growth upon subcutaneous or intratesticular inoculation into the appropriate hosts.

In view of the successful transplantation of this tumor to mouse embryonic tissues, an effort was made to transfer the tumor to guinea pig eyes in association with guinea pig embryonic organs. The transplanted material filled the anterior chamber in ten to 14 days, however, sectioned tissues of representative guinea pigs killed at this time revealed

only growing embryonic tissue and were completely devoid of Brown Pearce tumor. Yet, after a latent period of several weeks, growth again occurred in the rabbit's eye which histologically proved to be the tumor in question. It would appear, therefore, that isolated viable tumor cells were present in the transplants and were unrecognized. The isolated cells apparently failed to survive over 30 days in the alien host as subsequent growth in the donor's eye was not accomplished after that limit.

The point of concern was not the success of heterologous transplantibility but rather its failure in certain circumstances and the unusual behavior of transplants in the anterior chamber of other species. The constitutional differences of various hosts suggested a casual relationship as exemplified by the sharply defined range of transplantibility with inclusion of the mouse, hamster and rat and exclusion of the guinea pig. It was again reiterated that this paralleled the vitamin C metabolic patterns in these species.

Greene's hypothesis has been seriously challenged by the observations of Lushbaugh and Steiner (1949) related in a report on intraocular transplantation of malignant lymphomas of the mouse, dog and man in heterologous species. In no instance was success attained in transplanting tumors from these donors to alien species.

A descriptive pattern of a transplanted tumor contained in this report is present herewith. "Typically the appearance of the piece of tissue and of the anterior chamber followed a well-defined course. Twenty-four hours after transplantation the aqueous humor was hazy and the color of the piece of tissue was fading. By three days the conjunctiva at the limbus was hyperemic and the transplant was dull gray or yellow. At seven days the piece was about one-half its original size, occasionally it could be found no longer. The mild ophthalmitis was usually resolved by this time. By 14 days the pieces of tissue were about one-third the original size and ivory or light gray.

The pieces decreased very little in size during the next five weeks, although an occasional piece disappeared entirely. At the end of this time the tissue was minute and almost pure white. During the following months no changes were noted in its appearance. An occasional eye developed suppurative panophthalmitis. In most cases the transplants in the eyes of guinea pigs were absorbed faster than did those in the eyes of rabbits. In rabbits, during the first 48 hours, it was not unusual for a definite gray halo to develop around the transplant. Histologic study of such a halo showed that it was composed of fibrin; most of the lymphocytes and granulocytes had died or disappeared from the transplant at this time.

At two days, the pieces were almost completely necrotic; they were eosinophilic and almost structureless, most of the cells were dead. Transplants still contained minute foci of nuclear debris at four days and contained many proliferating fibroblasts. Transplants were composed of condensing collagenous connective tissue. Lymphocytes and plasma cells were present."

These observations, having demonstrated that malignant lymphomas did not grow following transplantations into ocular anterior chambers of alien species, summoned a refutal of Greene's autonomous theory of malignancy. In the first place, the invasiveness and metastasizing ability of the malignancies were high as evidenced by the short course of the diseases in the donor and generalized distribution at physical examination or necropsy. However, the fact that regression proceeded rapidly unless the tumors were placed in the eyes of the homologous strains set this group apart from neoplastic diseases.

An effort was made to explain heterotransplant failures on other bases than nonmalignancy and/or absence of autonomy. The deduction was made that either there existed non-neoplastic diseases whose manifestations were indistinguishable from those of neoplasms or that successful intraocular transplantation in alien species was inadequate as a test for malignancy. The host could have died from the effects of a vigorously growing mass of typical cells from which there had

been dissemination and formation of secondary masses in other parts of the body, yet according to Greene, the disease was not necessarily an autonomous malignant neoplasm. Since it was an accepted fact that there were in tumors different degrees of malignancy, the investigators felt that no doubt there were also different degrees within the definition of autonomy.

The failures of takes was explained by the following points: (1) lack of resistance of these cells to various types of trauma and adverse conditions, (2) short life span of these cells, and (3) the rapid development of immunity.

The first premise of the discussion of the transplant failure concerned the cells of the hematopoietic system which were notoriously susceptible to injuries. The injuries were designated as temperature changes, radiations, adreno-corticotrophic and adrenocortical hormones, non specific heterologous body fluids, various drugs and metabolites. Cells of malignant lymphomas as contrasted with hardier cells of fibroscarcoma and certain other neoplasms were expected to die at a higher rate. It was indicated that insufficient numbers of cells might survive to produce a viable tumor. Obviously, successful transplantation depended on the survival of cells.

The second factor considered as affecting the survival was the natural life span of the cells themselves. Malignant lymphoma cells probably existed no longer than corresponding

normal type. If cells died because of short life span before a new source of nutrition was established, the transplant was absorbed. However, that this was not the only factor concerned was shown by growth of a malignant lymphoma of mice in homologous and heterologous strains.

An attempt was made to explain the failure of transplant growth on the basis of a rapid development of immunity. This statement was based upon two factors: (1) the aforementioned short life of cells, the shortest exhibited by any type of tissue and (2) after transplantation early necrolysis of some of the transplanted cells could have released antigen which quickly aroused a humoral defense reaction.

It was interposed at this point that according to Greene (1942) the anterior chamber was not isolated but it exhibited antibodies formed elsewhere. The presence of these antibodies in the eye could have augmented the non specific causes for cell death.

That immune reaction might be the determining factor in failure of heterotransplant was further substantiated by interesting work done by Lushbaugh and Steiner also reported in this same paper. A dog subjected to total body x-radiation a procedure which inhibits antibody production, was successfully inoculated with a canine lymphoma.

The foregoing explanation of heterotransplant failure was based upon a short life span coupled with an inherent

high degree of susceptibility to injury which forecasted early death for these cells. This early mortality, in turn, incited antibody formation by the host upon release of antigen from the dying cells. The point of the discussion was this, if antibodies could unfavorably influence intraocular transplants then the fate of the tumor would be less dependent upon the inherent autonomy and malignancy of cells.

These investigators indicated that this explanation might explain the successful takes of brain tumors, characterized by low malignancy but long cell survival, also, fibrosarcoma, chondrosarcoma and carcinoma of the prostate. Likewise, transplant failures of highly malignant tumors of childhood, described previously by Eichwald (1948), and malignant lymphomas both composed of short lived, labile, highly malignant cells, were attributed to immune response by the host.

The final comment contained in this work concerning the value of the anterior chamber was that it was no aid in diagnosing malignant lymphoma but has merit for studying the histology of certain cancers.

The transplantation studies of the renal carcinoma of the frog (Rana pipiens)by Lucké and Schlumberger (1940a, 1940b) and Schlumberger and Lucké (1949) were the most intensive studies on the characteristics of transplants of single neoplasms undertaken up to this time. An effort was made to gain understanding of the effect of repeated transfers

and prolonged maintenance in anterior chambers upon the tumor. Points of concern were the rate and manner of growth, malignant properties (invasiveness), response to changes in temperature and behavior when transplanted to alien species.

The incidence of takes over a period of 14 generations did not reach 100% until the fourth generation. During these successive generations no trend toward longer or shorter length of time to fill the anterior chamber was noted. The manner of growth was adequately described in a previous publication of these authors (1939).

The observations showed that at a higher temperature, 28°C., the rate of growth of the transplant was much accelerated, more rapidly vascularized, and the tubular growth tended to become cystic through accumulation of fluid. At 7°C. the growth was greatly retarded.

The most significant findings contained in this report pertained to the effect of serial transfer on subsequent heterotransplantation. Early experiments indicated that the renal carcinoma could be established in the eyes of other species of the same family of frogs. In a member of a different family (the toad) the proportion of successful transplants had been somewhat lessened. In animals of different classes of cold-blooded vertebrates (the goldfish) the transplants regressed. Furthermore, homologous passage prior to transfer to other hosts did not facilitate heterotransplantation.

Eichwald et al (1950) published results of an initial study on the significance of the anterior chamber in tumor transplantation. Although the work thus far reported was concerned with only homologous transplants, attention should be drawn to the fact of the increasing numbers of reports concerned with finding the limitations of the anterior chamber. This was contrasted to the studies wherein the objective was apparently to transplant successfully as many types of tumors as possible. In this work, an attempt was made to determine whether or not growth of a tumor transplanted to the anterior chamber was limited to this space or progresses beyond it in a host in which the same tumor would not if transplanted subcutaneously. Progressive tumor growth was significantly more frequent in mice with inoculations into the anterior chamber than the latter. Gross metastases were not observed with this particular strain of tumor, mouse neuroblastoma C1300.

The report of a thorough investigation by Morris et al (1950) also appeared the same year. The purpose of this study was to determine if tumors of human origin could be successfully transplanted into the anterior chamber of eyes of animals. The objectives of the study were: (1) whether the method could be used as an aid in diagnosis and prognosis of malignant disease, (2) what were essential factors for successful transplantation and (3) the biologic factors for growth of the transplant.

In this report, the following criteria were given for successful transplantation: (1) vascularization of transplant by the vessels of the host, (2) definite growth of the transplant within a reasonable period, (3) morphologic and cytologic similarity of the transplant and the tissue originally transplanted and (4) possibly, eventual metastasis.

Quite logically, since the results of this study offered little evidence that heterotransplantation of malignant human tumors could be accomplished, an attempt was made to standoff Greene's criticism of present tumor diagnosis. To reiterate, Greene (1948) censured the generally accepted concept of diagnosing neoplasms by their morphologic and cytologic characteristics. It was suggested that tissues should be assessed according to their behavior as transplants.

Morris attacked Greene's theory on the basis of practicability from a clinical standpoint. The argument was that even if infiltrative or metastatic tumors were transplantable to the anterior chamber of alien species, the clinical value of such a procedure was limited. The goal in diagnosis was to discover neoplasms while they were still largely or completely in situ, not after they were widely infiltrative or had given rise to distant metastasis.

A seemingly peculiar finding was reported in this paper concerning intralenticular transplants. Even benign tumors

survived when transplanted into the lens. One fundamental difference between anterior chamber and intralenticular transplants was the vascularization of the transplants in the anterior chamber as compared with the lack of vascularization of intralenticular transplants. The method for accomplishing this type of transplantation was not elaborated upon, and to this writer knowledge was without antecedent.

MATERIALS AND METHODS

A. Avian Lymphoid Tumor (Olson Tumor)

This tumor designated as RPL 12[1] is one of several strains of lymphoid tumors maintained at this laboratory. It was selected because of its rapid rate of growth when introduced into the pectoral muscle of the chicken. Serial passage of this tumor provided an abundant supply of fresh material.

The original tumor was isolated in 1937 from a field case at Amherst, Massachusetts. After thirty passages, Olson (1941a) reported studies on the transmission of this tumor. Growth was obtained in 67.7% of the birds inoculated. After growth was established, the tumor either eventually regressed, remained localized at the site of inoculation, or metastasized to visceral organs, insuring ultimate death. Regression was observed in 44.3% of chickens in which implants developed. In 38.7% of the cases in which growth took place, the tumor remained localized at the site of inoculation. It should be noted that metastasis occurred in only 17% of all birds in which growth was observed.

This tumor strain was obtained for use from Dr. Olson at the 138th serial passage in 1942. Through the 140

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[1]Abbreviation for Regional Poultry Laboratory

passages conducted at this laboratory since that time, increased malignant tendencies of this strain have been observed.

At this point it should be emphasized that the number of cells per inoculum was standardized, otherwise conclusive data could not have been derived from this extensive passage work. Whereas 29.1 days was the average survival time for birds inoculated in the first ten passages, between the 31st and 40th passages the interval dropped to 8.2 days. This interval has remained approximately the same during the last one hundred passages. Likewise, the percentage with pectoral muscle tumor only decreased from 37% in the first ten passages to 4.5% average for the 31st to 40th passages. These figures indicated that nearly every implant metastasized[1] and that the infiltration of neoplastic cells into various organs of the chicken was strongly correlated with the average survival time. Another significant point was that a tumor developed in nearly every case in most passages.

Statistics compiled at this laboratory indicate that the organs showing metastatic lesions were many, however, the percentage of involvement was worthy of note. Compilation of figures during the 140 passages showed that the

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[1] The word metastasis is used in a broad sense here. There is some concern among workers as to the pathogenesis of these transplanted cells in the avian host.

liver was positive in 84.8% of the cases. The spleen was involved in about two-thirds of the cases (66.2%). Following this the percentages were: kidney 35.2%, gonads 27.2%, adrenal gland 13.3%, proventriculus 11.5%, heart 7.6%, and pancreas 6.8%. Infiltration into nerves as shown by gross examination was found in only 0.65% of the cases.

The foregoing data concerned the malignant properties in the homologous host of the tumor used in this investigation. No average survival time figures were available for the passage made in conjunction with the present work. This was not possible as the material was harvested as soon as sufficient growth occurred to make transplanting practical. The reasons for reviewing these properties was to emphasize the high malignant potency of this strain in the chickens maintained at this laboratory. These infiltrative qualities arose from a field case with which Olson had difficulty in transplanting successfully in the early experimental attempts.

Another ramification of study using this tumor was reported by Burmester et al (1944). By inoculation of small numbers of cells at various sites a higher percentage of survival could be obtained. This report stated that a certain degree of immunity was induced by the regressing tumor. Birds implanted with tumor cells by any one of several routes were found to be immune to a second inoculation by other routes. Immunity induced by a small number of neoplastic cells could not be overcome by subsequent inoculation with

as many as 10,000 times the number used in the first inoculation. This immunity extended for a relatively long period (at least 202 days).

B. Propagative Methods

The objectives of propagation of RPL 12 were:

1) to have available at frequent intervals an abundant supply of fresh tumor.
2) to contrast the variations, if any, culminating from material from a different source, namely, chicken embryo propagation.
3) to maintain microbial sterility at all times.

1. Procedures used for propagative passages in chickens.

Pedigreed single comb white leghorn chickens of line 15[1] maintained at this laboratory were used in all experiments concerned with the present work. The birds were selected from replacement stocks used for other experimental studies. All birds used had been hatched the same year, except in one passage it was necessary to resort to two year old male birds. These birds were maintained in batteries either singly, in pairs, or in groups of six, depending upon the capacity of the battery available during the propagative period. No special attention was warranted during the passage period.

[1]Line 15 is considered genetically susceptible to lymphomatosis; that is, more so than certain other lines maintained at this laboratory.

At the time of harvest, the birds were taken alive to the necropsy room of the laboratory and electrocuted. This was followed by dipping the bird into hot detergent bath which facilitated picking and washing the breast. The skin over a large fraction of the breast was swabbed with zephiran[1] and with sterile instruments was incised over the tumor. More zephiran was applied to the bared muscle. With a second set of sterile instruments, the pectoral muscle tumor was then incised from its sternal attachments and placed in a sterile petri dish. The bird was subjected to post-mortem for verification of metastatic lesions.

Usually the inocula were prepared immediately, however, on occasion it was held under refrigeration (35°F.) for several hours. During the course of this study, the harvested transplant was put to several uses[2], 1) minced and reinoculated into chicken breast muscle or introduced onto the chorioallantoic membrane of chicken embryos, 2) transplanted into the anterior chamber of guinea pigs or, 3) placed in ampoules in the frozen tumor stock for future reference.

A portion of the harvested tumor was weighed in a tared test tube to obtain a rough estimate of the dilution needed

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[1]Tincture of zephiran chloride 1:1,000, a brand of benzalkonium chloride refined and manufactured by Winthrop-Stearns Co., Rensselaer, New York.

[2]The procedures described were standard methods employed at this laboratory and were adopted before the present project was started.

for cell suspension. The actual mincing was accomplished by using a hollow cylindrical instrument with a screw top plunger, first described by Olson (1941b). The dilution of the cell suspension was generally one part of tumor to four parts of sterile (0.85%) saline. No attempt was made to standardize the number of cells per inoculate. Penicillin G and streptomycin were incorporated in the suspension as bacteriostatic agents.[1] Every suspension was checked for bacterial sterility by incubating on 5% tryptose blood agar plates for 72 hours. Once the cell suspension of neoplastic cells was made, it was generally placed into the site of inoculation within the hour.

Preparatory to inoculation each bird was swabbed with zephiran over the left breast muscle. The (1.0 ml.) injection of tumor cell suspension was placed between the superficial and deep pectoral muscles close to the anterior part of the keel.

2. Procedures used for propagative passages on chicken embryo chorioallantoic membranes. The method employed was essentially that described as the standard technique of chorioallantoic inoculation by Beveridge and Burnet (1946) and method three of Cunningham (1948). Essentially it involved

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[1]An inoculum was generally prepared from 5 gm. of tumor and 18 ml. of (0.85%) sterile saline, to which were added 1 ml. each of penicillin G and streptomycin from stock dilutions. Final concentrations of the antibiotics were: penicillin G--500 units/ml. and streptomycin--0.005 mg./ml.

the production of an artificial air cell thus allowing a more certain approach to the chorioallantoic membrane than other procedures.

The eggs used in this work were obtained from birds maintained at this laboratory. They were set in a "Humid-aire"[1] rotary electric incubator of 300 egg capacity with a hatching unit below. Temperature and humidity were adjusted according to specifications. Initial candling was done between the fifth and seventh days of incubation.

Since this avian lymphoid tumor required a relatively long period of the embryonic life of the chick to develop, eggs were candled on the nineth and tenth day of incubation. Those containing a living embryo were inoculated with 0.05 ml. of the tumor suspension. This suspension of neoplastic cells was prepared the same way as described previously for chicken breast muscle implants. In all passages it was prepared from a fresh tumor, never frozen material.

Following inoculation, the eggs were placed in the hatching unit below in a horizontal position and were never rotated. The humidity was lowered to allow greater loss of fluids from the eggs which is more conducive to the formation of a large air space. The eggs were again candled on the

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[1]Manufactured by the New Madison Incubators, Inc., New Madison, Ohio

second and third day following inoculation for elimination of those dying from non-specific causes.

On the eighth or ninth day following inoculation the eggs were opened in an ultra-violet light cabinet and the tumors asceptically lifted from the chorio-allantoic membranes into sterile petri dishes.

C. Heterologous Transplantation

1. Technique for anterior chamber inoculation. The technique, employed during the present study, was essentially the same as described by Greene (1941a). This process was entirely mechanical; the task being one of placing a piece of tumor in the anterior chamber of the eye. Once the technique was mastered, it was a relatively simple thing to do, requiring less than one minute to perform.

During the course of the study it was found that the following points were worthy of concern when undertaking a method such as this.

1. Some degree of anatomical knowledge[1] of the eye, both microscopic and gross was essential. The relationship of the curvatures of the eye-ball to straight implanting instruments can be learned only through experience.
2. A method for relative immobilization of the eyeball

[1] The Vertebrate Eye by G. L. Walls (1942) was helpful in this study.

was necessary.

3. The process was kept as free of bacteria as was possible.
4. The continuity of the sterile anterior chamber was interrupted where the least inflammatory reaction was provoked.
5. Satisfactory topical anesthesia was enforced.

Guinea pigs, the heterologous host in the present study, were obtained from colonies maintained by the Michigan Department of Health. During the course of the study, the diet consisted of prepared pellets; occasionally green leafy vegetables were available. Fresh water was supplied at all times. No special care was given in addition to that which was customarily employed at an experimental animal house of this laboratory. Although both males and females were inoculated, care was exercised in allowing only one male per pen.

Butyn sulfate[1] (2%) was employed as the topical ophthalmic anesthetic instead of cocaine in an attempt to lessen compensatory post anesthetic inflammation. Satisfactory anesthesia was produced in approximately ten minutes by two applications of this drug. Mercuric chloride (1:10,000) was instilled in the eye during this waiting period as a bacteriostatic agent.

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[1] Manufactured by Abbott Laboratories, North Chicago, Illinois

When sufficient insensibility had been produced, the eye was immobilized by applying pressure below it with the index finger. The incision, 1-2mm. in length, was made dorsally at the limbus with a Graefe corneal scalpel; care being exercised to avoid scleral blood vessels. The slight amount of aqueous humor which seeped out as the blade was withdrawn released some of the internal pressure of the anterior chamber. Small pieces of tumor, 1-2 mm. in diameter, were manipulated into a 20 gauge, 1½ inch sterile needle by gently brushing the bevel across a larger piece of tumor held in a forceps. This process was more easily accomplished with pectoral muscle tumor than chicken embryo propagated tumor because of a difference in texture. A stilet corresponding to the needle size was placed in the hub end. The tumor particle was then released in the inferior angle of the iris by operating the stilet as a plunger. Once the transplanting needle was withdrawn the tumor was further manipulated, if needed, by stroking the surface of the cornea with a blunt instrument. Generally any hemorrhage occurring during the procedure was negligible. The corneal incision healed in a few days leaving little evidence of the operation.

2. Method of transferring anterior chamber contents reciprocally to chickens. The following method was employed to study the length of survival time of neoplastic cells in the

anterior chamber of the eye of the heterologous host. After extirpation, the eye was placed in a sterile petri dish containing 0.85% saline, to which antibiotics had been added, for approximately thirty minutes. The eye was then placed in a sterile mortar where the anterior part was excised from the other ocular structures. The contents of the anterior chamber were diluted with the same diluent as mentioned above so that single ml. injections could be made into the chicken breast muscle.

D. Histological Methods

1. Perfusion technic of fixation. The most reliable basis for verifying the destiny of transplanted tumor cells was microscopic sectioning. Since the eyeball is difficult to handle histologically, special techniques were required to obtain satisfactory results. The fixation process used in connection with this study was suggested by Dr. A. M. Lucas[1].

The guinea pig was injected intraperitoneally with 0.5 ml. of nembutal[2]. This was supplemented with ether inhalation to effect more quickly a deep state of anesthesia. Each guinea pig also received approximately 13 mg. of sodium nitrite intraperitoneally.

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[1] Histopathologist at the Regional Poultry Research Laboratory

[2] Commercial form of sodium pentobarbital, manufactured by Abbott Laboratories, North Chicago, Illinois

When the proper degree of narcosis was achieved, the subject was placed on its back in a wooden trough and restrained by tying one foot to each corner. After removing the ventral thoracic wall, the pericardial sac was incised at the base of the heart to facilitate a rapid approach to the aortic trunk. A ligature was placed around it, but not tied. A small incision was made partially through the myocardium of the left ventricle. Through it a glass cannula was passed into the ascending aorta. When the proper position for the cannula was located, the ligative was tied, holding it in place. The dorsal aorta was clamped off to conserve fixative. Ringer's solution was introduced first to clear the vessels of blood constituents. The anterior vena cava was opened so that a continuous circuit could be established, insuring a proper rate of flow. Care was exercised to keep air bubbles in the conducting tubes to a minimum. During the entire process the pressure in the lines was maintained between 135-150 mm. Hg[1] by means of a rubber bulb. Both the Ringer's solution and the fixative were introduced at the approximate body temperature of the guinea pig.

When the submaxillary veins appeared free of blood elements; Kolmer's fixative was introduced from another

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[1]This figure was estimated as closely approaching the mean arterial blood pressure from data given in The Physiology of Domestic Animals by H. H. Dukes (1947).

branch in the conducting lines. More favorable results were obtained when the clamps on the tubing were adjusted to give a slow steady flow of fixative, maintained for about twenty minutes. Clipping off the end of the tongue proved a reliable index by showing the degree of fixation. Upon completion of the perfusion process the eye, carefully enucleated, and trimmed of extrinsic muscle attachments, was placed in more of the same fixative for 24 hours. The components of Kolmer's fixative as given by Walls (1938) were mixed just before using in the following concentrations:

potassium bichromate (5%)---------- 4 parts
formalin (10%)---------- 4 parts
glacial acetic acid---------- 1 part
trichloracetic acid (50%)---------- 1 part
sat. aq. uranyl acetate---------- 1 part

2. Embedding, sectioning and staining. The hot celloidin embedding technique as described by Walls (1932) was followed in the preparation of eyes for histological study. After a water wash of 24 hours and dehydration through four levels of alcohols (35%, 50%, 70%, 90%), the eye was passed into celloidin. Five concentrations (2%, 4%, 6%, 10%, 14%) were prepared from collodion cotton dissolved in a 50-50 mixture of ether and absolute alcohol. An embedding oven was used to maintain the temperature at about 60°C.

Changes into higher concentrations were generally made at 24 hour intervals. This relatively rapid process was

made possible by vapor pressure maintained inside of the embedding bottles. Pyroxylin strips were added when the highest concentration was reached in order to thicken the mass. The selection of the proper time for hardening with chloroform required considerable judgement during the process.

The embedded blocks of celloidin were sectioned at 12-15 micra on a sliding microtome. Two difficulties were encountered with this process. The fixative had penetrated, in most cases, only halfway through the crystalline lens. As the blade passed through the block, the thin slices of tissues were often badly torn. This was alleviated by lifting the lens from its capsule, thus preparing slides without it. The other difficulty was the loss of many sections due to failure of the sections to adhere to the slide. As a consequence, the sections were stained in small dishes before mounting.

The following stains were routinely used: Mallory's aniline blue collagen, Masson's trichrome and Ehrlich's haematoxylin and eosin.

EXPERIMENTAL RESULTS

A. Propagation of the Avian Lymphoid Tumor RPL 12

1. In the pectoral muscle of chickens. This tumor was the only neoplastic tissue employed during the study. Infiltrative and metastatic properties of this strain have been previously given. No additional data were obtained concerning the propagation in the homologous host; the objective was to make available tumor for heterologous transplantation into guinea pig eyes.

During the present study, 15 serial passages involving 55 chickens were made. Usually three birds were inoculated during each transfer. Tumor for the first passage was selected from frozen stock maintained at approximately -65°F. with dry ice refrigeration. The production of tumor in that passage lagged; the time interval extended to 15 days before subsequent passage was made. The explanation given relative to this finding was that freezing destroys a very large percentage of the tumor cells. Even though the samples were "shock" frozen, apparently rupturing of the cells occurs.

The tumor was harvested at shorter intervals from seven to twelve days in the remaining passages. Since the pectoral tumors were taken when the size warranted harvest,

many of the inoculated birds showed no clinical symptoms during the passage interval. In several cases, females continued to lay until the tumor was harvested. Some indication, however, for the proper time of collection was ascertained by observing the bird. When the bird became listless and the fleshy comb and wattles paled, generally death ensued in 24-48 hours.

Tumors produced during the serial passages concerned with this study were used for several purposes. Some material was selected, in addition to that used for heterotransplantation, for embryonated egg inoculations, tissue culture and precipitation test antigen studies.

2. On chicken embryo chorioallantoic membranes. This method of propagation was adopted to offer another source of tumor for heterotransplantation. The inocula for the eight sets of embryonated eggs used during the course of the study were prepared from fresh tumor. This tumor was taken from either chicken pectoral muscle passage or serial passage in embryonated eggs. Generally 20 eggs were inoculated for each passage.

Little difficulty was encountered with this propagation procedure. The results of the first passage showed only one tumor produced as a result of macroscopic examination. Subsequently, the percentage of "takes" increased; the fifth passage resulted in tumor growth in every egg inoculated. The reason for this increase was not definitely known.

There was some indication that the neoplastic cells became adapted by serial passage in embryonated eggs. This fact has been partially substantiated by the unsatisfactory results obtained when the chorioallantoic membrane was inoculated with cells from the frozen tumor stock. However, little direct evidence was available due to the fact that the number of neoplastic cells per inoculum was not standardized.

Grossly, the tumors varied from approximately 2-15 mm. in diameter. The growths, varying in thickness from 1-3 mm. were depressed, for the most part, into the allantoic cavity. The high degree of vascularity associated with the growth apparently conferred the reddish brown appearance to the viable mass. Since both surfaces of the tumor were covered with glistening embryonic membranes a texture contrast from the pectoral muscle tumor was noted. Some embryonated eggs showed multiple foci of tumors; the individual focus was generally smaller in these cases.

From the microscopic viewpoint, the neoplastic cells infiltrated into the chorioallantoic membrane; the increased number of cells spreading the ectodermal and entodermal layers. This was verified by the low power photomicrograph (figure 1) of such a site. Anaplastic cells and the numbers of mitotic figures exhibited (figure 2) were considered typical of the RPL 12 strain.

One ramification of study was concerned with the pathogenesis of the neoplastic cells in the embryo. Several of the organs were prepared for histological study from embryos inoculated on the chorioallantoic membrane. The limitations of the present investigation presented no evidence of meta static lesions.

On some occasions chicks were hatching at the termination of a harvest. Preliminary investigation revealed that, other conditions being conducive, apparently the embryo was not greatly affected by the transplant, at least not immediately. The low state of humidity, however, made it difficult for any chicks to free themselves from the membranes.

B. Results of Heterotransplantation

During the course of this study, ten passages were made involving 76 guinea pigs. The initial passage consisted of introducing a cell suspension of RPL 12 into the right eyes of six guinea pigs. Control inoculations used were (1) heat killed cell suspension, and (2) normal muscle and lymphoid cell suspension; likewise injected into groups of six guinea pigs each. The neoplastic cells of the control were killed by heating the suspension in a water bath at 56°C. for 30 minutes. The muscle and lymphoid cells for the second control group were obtained from chicken embryo pectoral muscle and thymus gland respectively.

Observations with an ophthalmoscope the first day after inoculations were made showed that the reactions incited in

the eighteen guinea pigs appeared much the same in all three groups. The most evident changes were:

1. cloudiness of the cornea, though a variation between individuals was noticeable.
2. active hyperemia of the iridic vessels.
3. prolonged dilation of the pupil in some instances, possibly due to the contraction of the dilatator muscle of the iris.
4. injection of the vessels at the limbus especially those near the site of the inoculation.
5. edema of the palpebral conjuntiva in some guinea pigs.

Examination of the three groups on the third day following inoculation revealed that some of the eyes were more cloudy. A larger number of inoculated eyes, however, were showing a greater degree of clearness and there were indications that the cloudiness was becoming patchy.

By the eighth day, several of the inoculated guinea pigs from all three groups showed a haziness in the pupillary space in apposition to the lens. Histological sections disclosed this reaction to be a lenticular degenerative process. The eyes of some of the guinea pigs appeared to return to normal; at least no evidence of the inoculation was visible. These variations were seen in both control groups as well as the group which received the live tumor suspension.

After an indefinite period of time, a relatively few individuals of this experiment and subsequent passages showed a condition resembling hypopyon. Ultimate rupture of the cornea was the fate generally associated with this condition.

All the remaining nine passages were undertaken by using a tumor fragment transplant rather than a cell suspension inoculum. The gross description of fragment transplant in the eye varied somewhat from the foregoing reactions incited by cell suspension inoculation. Generally a few hours after inoculation, a small cloudy area formed about the transplant. This was followed by the development of a membranous-like plaque which in some cases completely enveloped the mesothelial lining of the cornea. Progressive development of this reaction, as observed with the ophthalmoscope was deceiving. Certain observations appeared to show the papillary development of the tumor as described by Lucke and Schlumberger (1939). After a variable period of three or four days gradual dissolution of the opacity became apparent. However, in the area most closely related to the transplants this cloudiness often remained for a considerable length of time. In other eyes, the opacity cleared after a comparable period, allowing excellent observation of the transplant. No evidence was obtained from daily gross observations that any proliferative change of the transplant occurred. The transplant remained visible in

some inoculated eyes for several months. Occasionally the transplant would disintigrate and apparently was absorbed by the host.

Evidence of vascularization was obtained on only two occasions. One inoculated eye showed clearly an extension of an iridic vessel across the pupillary space to the transplant. Subsequent sectioning of this eye revealed no viable transplant. Another guinea pig showed that capillary loops of the anterior stroma of the iris proliferated to form anastomosing ringlets around the pupillary margin.

Apparently, the reaction incited in the host by the transplant was localized. This assumption was made from daily temperature data of the inoculated animals, as no deviations were noted from the normal body temperature of guinea pigs.

Control inoculations of chicken embryo pectoral muscle and spleen tissue fragments provoked a similar reaction.

From a histological point of view, at no time during the present course of study, was anything but a severe inflammatory reaction inferred from slide preparations. One guinea pig was perfused daily from a particular passage so that the reaction could be studied at 24 hour intervals. Certain patterns of reactions on the part of the host were often revealed in the inoculated eyes. The salient features of this reaction appear below.

1. The initial cloudiness of the cornea was due to cellular infiltration of leucocytes, primarily polymorphonuclear phagocytes and edematous distension of the stromal fibers. (figure 3)
2. The reaction of the host tended to "wall off" the transplant in the anterior chamber, mostly with fibrin and albuminous coagulation. (figures 5 and 6)
3. The transplant very early, often in two days, underwent degenerative changes. The acidophilic staining transplant generally revealed that massive hyalinization had taken place. The transplant was invaded peripherally by inflammatory cells of the host. (figure 4)

Though viable tumor cells must have lived at least seven days in the anterior chamber as demonstrated by later study, none were detected from histological examination.

C. Data Concerning the Survival Time of Neoplastic Cells in the Anterior Chamber of the Guinea Pig Eye

The description of the technique employed during the study was included in another section of this thesis. The contents of the anterior chamber of a sixth passage animal were diluted so that 1 ml. inoculations could be made into the pectoral muscle of three chickens. The guinea pig had been inoculated with a tumor fragment seven days previously.

No palpable growth was evidenced until 15-18 days after the three birds had been inoculated. The birds exhibited no clinical symptom of disease during this period.

In order to obtain some information concerning the duration and pathogenesis of these tumors, the birds were not sacrificed at the same time. The first bird, destroyed on the fifteenth day following inoculation, displayed a small pectoral tumor, mostly necrotic. No gross metastatic lesions were observed. After holding a second bird four days longer, necropsy findings revealed a few scattered white foci (2-4 mm. in diameter) in the liver. These were shown histologically to be neoplastic lymphoid areas. No lesions were found in other organs. As it was necessary to conclude this study, the third chicken was killed seventeen days after the first bird or thirty-five days after inoculation. A small caseous core remained at the site of inoculation and no metastatic lesions were noted.

Figure 1. Infiltration of neoplastic cells (RPL 12) between the ectodermal and entodermal layers of the chorioallantoic membrane of a chicken embryo. (65x)

Figure 2. Cytological appearance of RPL 12, showing anaplastic tendencies and several mitotic figures. (1400x)

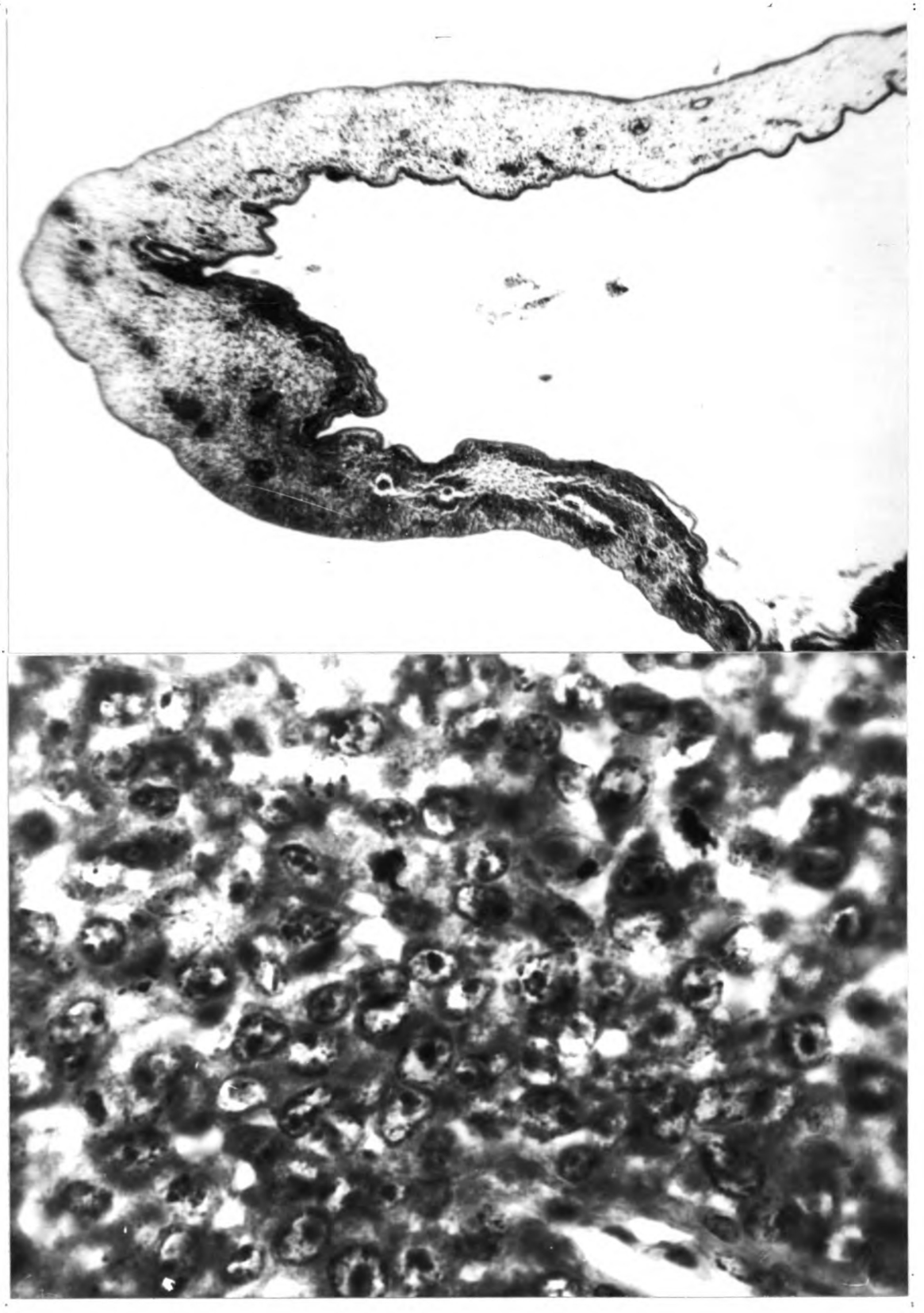


Figure 3. Cellular infiltration of leucocytes and edematous distension of the stromal fibers of the cornea. Typical of the early inflammatory response in the guinea pig eye. (220x)

Figure 4. Massive hyalinization of the tumor fragment with peripheral invasion of inflammatory cells of the host. (65x)

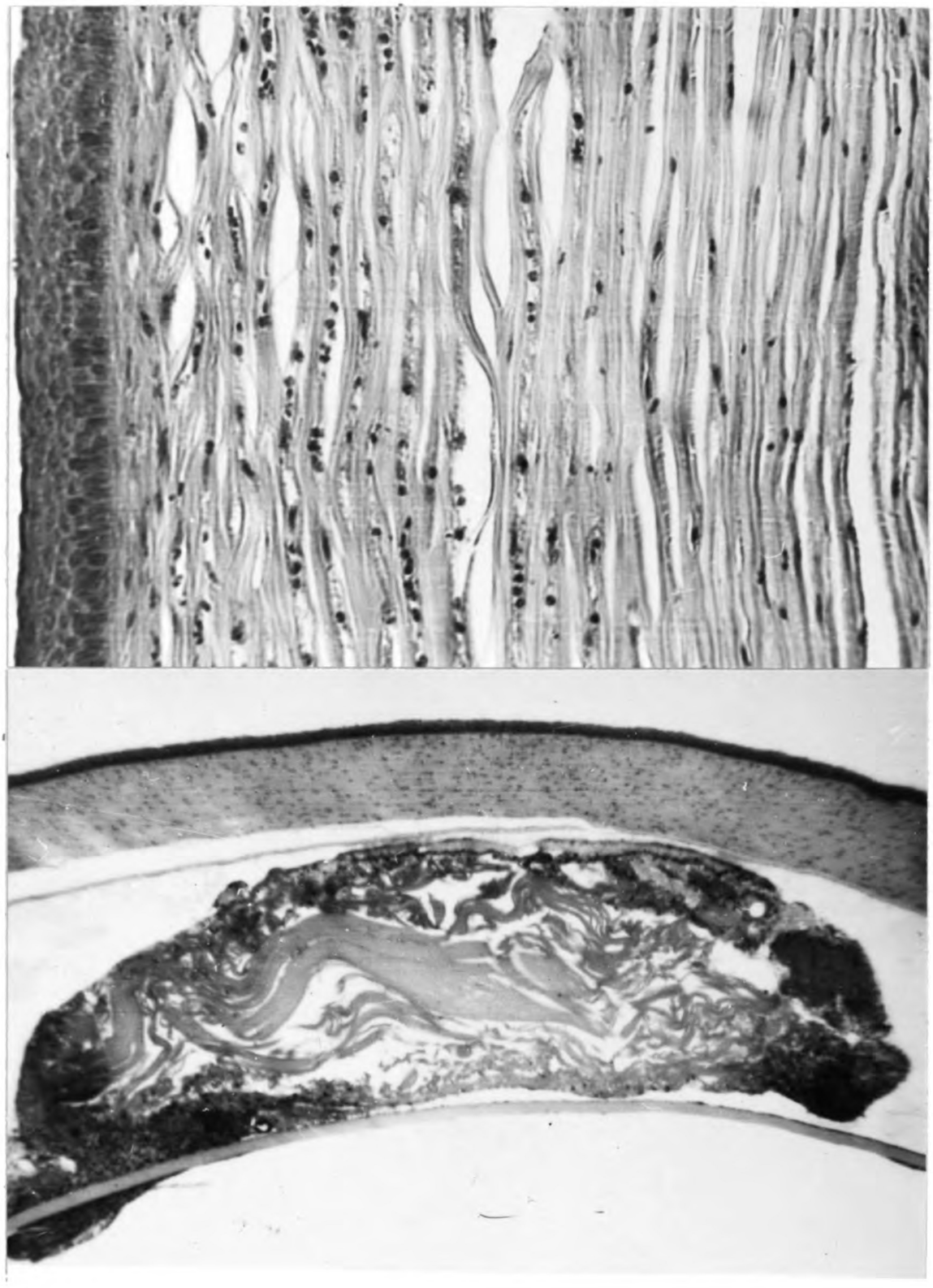
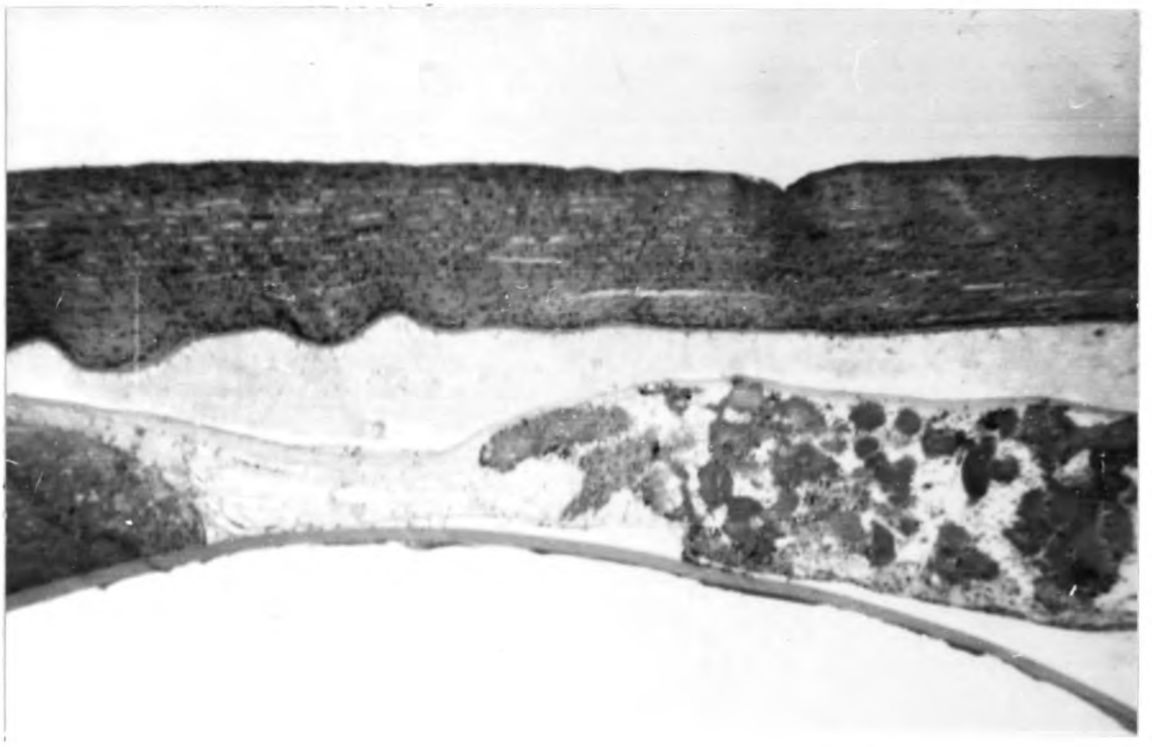
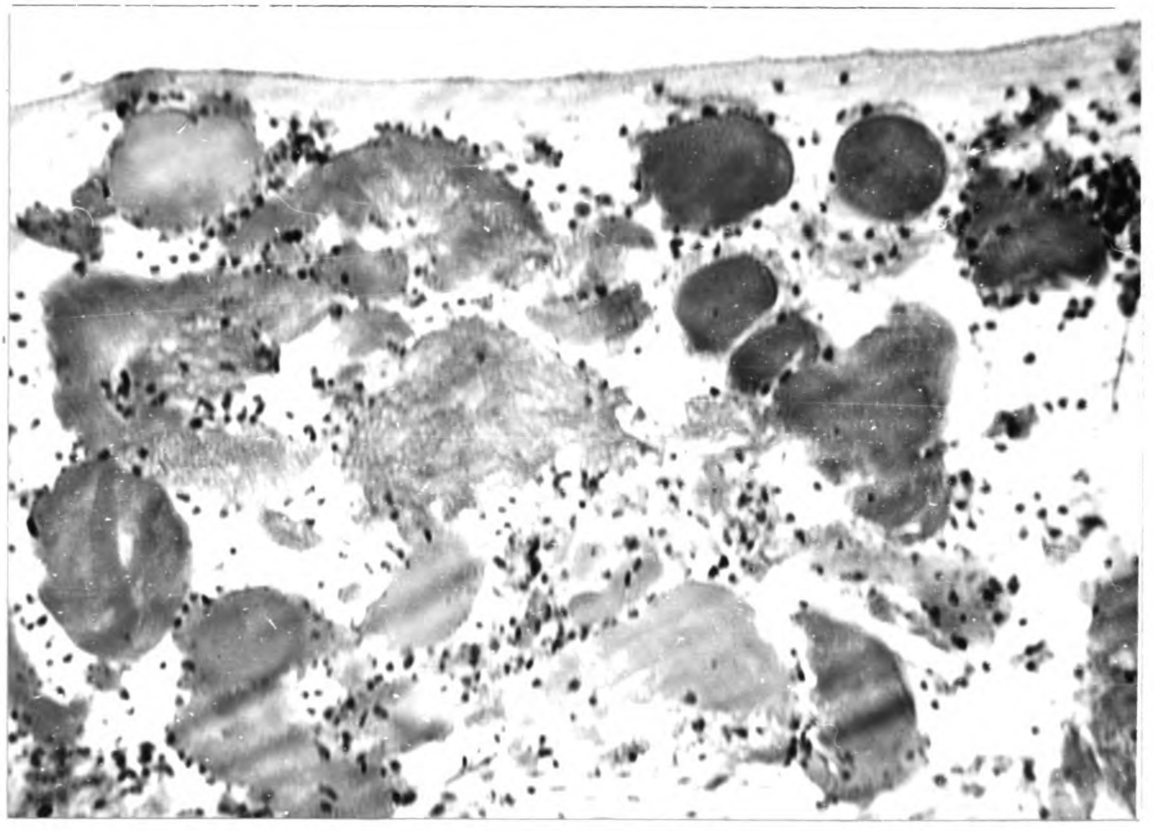


Figure 5. Host reaction showing the "walling off" of the tumor fragment mostly with fibrin and albuminous coagulum (65x)

Figure 6. Higher magnification of the above figure demonstrating the severity of the host response. (220x)





DISCUSSION

During the study encompassed by this thesis several aspects confronted the author. The prime objective concerned the heterotransplantibility of an avian lymphoid tumor. The discussion of experimental data will be limited to this topic.

The experimental results conclusively showed that none of the criteria for successful transplantation as given by Morris et al (1950) has been manifest in this present study. Transplants of an avian lymphoid tumor strain, RPL 12, failed to produce a semblance of growth. Instead, a severe inflammatory reaction of the anterior chamber ocular was incited.

From the reports of Shrigley et al (1945, 1947) adequate evidence was available to show that successful transplantation of certain avian tumors into guinea pig eyes had been accomplished. This fact seemed to indicate that the donor and the host exercise less effect than the inherent properties of the tumor upon the viability of the transplant. The avian tumors transplanted successfully in guinea pig eyes by Shrigley included the Rous sarcoma, two spontaneous fibroscarcomas and three chemically-induced tumors. This group contained filtrable as well as non-filtrable growths. No mention was made of the heterotransplantation of a lymphoid tumor.

As has been suggested by Lushbaugh and Steiner (1949) and a description of RPL 12 in this paper, the invasiveness and metastasizing ability of lymphoid tumors were high as evidenced by the short course in the homologous host and the generalized distribution at necropsy. The histological pattern of such rapidly growing tumors was landmarked by a high percentage of the cells undergoing mitosis. Since tumors such as these are among the most susceptible to roentgen rays, it seems reasonable that neoplastic cells exhibit a greater lack of resistance during cellular division than at other times during the life of the cell.

The fact is generally recognized that the cellular elements of the blood have a relatively short survival time as compared to the other somatic cells. As the normal lymphocyte is apparently the progenitor of the lymphoid neoplastic cell, the assumption is that the average life span of the tumor cell is approximately the same as the normal cell. This is axiomatically substantiated by the correlation of living things with rapid metabolic rates with short survival times.

Gross and microscopic examination of tumor harvested at the shortest passage interval (seven days) showed massive central necrosis where the cells were the oldest. This may be partially accredited to the poor exchange of metabolites in the core of the growth. From a quantitative standpoint, many cells of a transplant have lived a large fraction of their span and thus, survive only a brief time in

the anterior chamber. Greene (1941) stated that transplanted cells must live for a period of time in the manner of tissue culture. During this interim the transplanted fragment persisted as a free entity deriving nourishment from the fluid of the chamber and its cells remained entirely independent of the tissue of the host. Since it is imperative that the cells survive to obtain a viable transplant, the average survival time of these neoplastic cells may be an important factor.

Greene (1945) stated that as the malignant tendencies of a tumor were evolved, the cells became independent of factors peculiar to the primary host. They gained the ability to survive and grow in their absence, thus approaching an autonomous state. It was contended that malignancy was free of all genetic and species barriers. The results of this study do not substantiate this hypothesis. This writer believes that the absolute standard of Greene does not apply to the present conditions. The difficulty lies with the infinite variations of nature; each living thing is an "island unto itself". Each higher living organism according to Loeb (1945) possessed a differential factor peculiar to itself. Most cells, however, are capable of some degree of modification, the less specialized cells more so than their more highly differentiated counterparts. Successful heterotransplantation is, therefore, most likely dependent upon the differential barriers which exist between

the donor and the host and the inherent characteristics of the tumor.

Since Greene (1942) had previously stated that the anterior chamber is not isolated but exhibits antibodies formed elsewhere, a plausible explanation for the immunity developed by the host against the transplant was suggested by Lushbaugh and Steiner (1949). Working with malignant lymphomas, it was suggested that heterotransplantation failures might be due to an immune reaction stimulated by the early mortality of the transplanted cells. Disintegrated cells were thought to release more antigenic agents than living cells. Since a review of literature revealed no transplanting attempts of neoplasms more closely related to avian lymphoid tumors than these malignant lymphomas, it is reasonable to assume that a similar pattern of heterotransplantibility might be exhibited.

The ability to synthesize vitamin C was suggested by Greene (1945) as a possible explanation for the ease of transplantation between mouse and rabbit as contrasted with the difficulty associated with tumor transfers from these species to the guinea pig. Since, according to Sherwood and Couch (1948), the chicken does not require vitamin C in the diet, it is more like the mouse and rabbit in this respect. This might have suggested a partial explanation for the transplant failures in this study had not Shrigley (1945, 1947) successfully transferred six avian tumors to the guinea pig.

eye. The question arises as to what affect the site of synthesis has upon the nutritive content of the anterior chamber.

Although the cells could not be demonstrated microscopically, it has been shown by injection of the anterior chamber contents of a guinea pig, inoculated seven days previously, into chickens that a certain percentage of the cells did survive. Pectoral tumors have been produced in chickens by Mack (1950) after the neoplastic cells had been carried as long as twenty days in the guinea pig eye. The original inoculum consisted of a cell suspension rather than a tumor fragment. Rous and Murphy (1914) stated that a greater percentage of the cells inoculated individually or in small aggregates would survive than those comprising a piece of tumor. With reference to the anterior chamber, possibly the explanation lies with the fact that individually suspended cells are afforded greater gaseous and nutrient exchanges than those of a mass.

Studies conducted at this laboratory by Burmester (1944) have shown that a few neoplastic cells will produce a breast tumor almost as soon as an inoculum consisting of 10,000 times as many cells. Tumor cells held seven days in the anterior chamber of the guinea pig before transplanting back to three chickens required a longer time (15-18 days) to produce a pectoral muscle tumor. The small growth regressed in ten days to two weeks and necropsy examination of two of

the inoculated birds revealed none of the metastatic lesions of RPL 12. A few lymphoid foci were found on the liver of the third bird. These results seen to indicate that the surviving neoplastic cells have undergone some alteration.

Since the cells that survived were apparently altered it might be assumed that the associated virus was likewise attenuated. Shrigley et al (1945) reported variation of the Rous sarcoma virus following growth in the guinea pig eye. Although studies to determine this have not been undertaken as yet, this possibility seems to offer the most promise for future study of heterotransplants of RPL 12. It is not unreasonable to assume that such an alteration might lead to the development of birds immune to lymphomatosis.

As to the field of intraocular transplants in general, this writer strongly feels that routine inoculation of a heterologous host will not replace the accepted concept of morphological and clinical diagnostic producers of neoplasms. The limiting factor, above all, is the uncertainty and variability of life itself.

SUMMARY

1. Fragments of an avian lymphoid tumor (RPL 12) were transplanted in the anterior chamber of the eye of 76 guinea pigs. Ten guinea pig passages were made during the course of the study.
2. Inoculated eyes were fixed by a perfusion technique, celloidin embedded and sectioned on a sliding microtome.
3. All heterotransplantation attempts failed to reveal histologically any evidence of tumor survival and growth.
4. The short life span of the neoplastic cells which influenced survival time in the anterior chamber and subsequently incited antibody response in the host were given as possible explanations of the results.
5. Implantations of the contents of the anterior chamber of a guinea pig eye, seven days after inoculation, into the pectoral muscle of chickens indicated possible alteration of the tumor (RPL 12).

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