as also observed in human beta-2A-type myelomas(20). 6) The peculiar type of large light cells was consistently found to be associated with the leukemia described here, in contrast with the cytological findings in ordinary gamma-type mouse paraproteinemias. Similar cells have been found to be pathognomonic of human beta-2A-myelomas (14). The degenerative lesions in cardiac and skeletal muscles, so far of unknown etiology, have not been observed in other types of mouse leukemia.

Summary. A transplantable mouse leukemia with the following characteristics is described: Production of a paraprotein of beta-2A-type. Presence of peculiar large light cells ("flame cells") in the leukemic infiltrations and degenerative lesions in cardiac and skeletal muscles.

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Heterologous Transplantation of Two Tissue Culture Lines of Glioblastoma Multiforme (TC 178 and TC 224).* (26711)

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It is extremely difficult to distinguish between "normal" and neoplastic cells growing in monolayer tissue cultures on a morphological basis(2,5). Furthermore, the histological features of neoplastic tissue culture cells do not give accurate information about the tumor of origin. It was thought that heterologous transplantation of tissue culture cells might solve these problems. The present communication deals with the successful heterologous transplantation of 2 permanent lines of human glioblastoma multiforme cultured in this laboratory. Glioma TC 178 has been growing since Sept. 4, 1957, and has been reported in detail in this journal(3). The line TC 224 has been in continuous cultivation since Feb. 28, 1959. A

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method of heterologous transplantation was utilized which is described in detail elsewhere (4).

Materials and methods. A. Tissue culture procedure. TC 178 and TC 224 were isolated from 2 different cases of human glioblastoma multiforme. The method of culture was the same in both instances. Fresh tumor tissue was minced with uterine scissors and washed several times with balanced salt solution. Small quantities of the mince were placed into several 8 ounce wide-mouthed Blake bottles. Twenty cc of culture medium (composed of Eagle's medium, glutamine† 1%, penicillin 100 units/cc, and streptomycin 100 mg/cc), containing 10% horse serum† were added to each bottle. The bottles were placed in a 37°C incubator. Growth was observed around the explants by the second day, and after 3 to 4 weeks all bottles showed a luxuriant growth. At this time the original medium and the floating necrotic debris were discarded and fresh medium was added. When the surface of the bottles was completely covered with cells, these were scraped with a rubber policeman, centrifuged at 500 rpm for approximately 10 minutes, resuspended in fresh medium and planted in new bottles. At present the medium of the cultures is changed at weekly intervals and new bottles are implanted with tissue culture cells at the same time.

B. Transplantation procedure. 0.03 ml and 0.1 ml of a suspension of 5×10^6 cells/ml of TC 178 or TC 224 were injected intracerebrally into untreated mice and guinea pigs respectively. Approximately 5×10^6 cells of glioma TC 178 or glioma TC 224 in 0.5 ml volumes were injected into the right flank of hamsters which had received, prior to inoculation, 200 r of total body radiation. The animals also received 4.5 mg of cortisone acetate on alternate days for 3 doses. Eight to 15 days after inoculation with TC cells, the animals developed subcutaneous tumors of varying size up to 1 cm in greatest dimension. The growing tumors were removed sterilely from the hamsters; parts were taken for histological examination and the rest cut into pieces approximately 3-4 mm in diameter. These fragments were transplanted into the anterior chamber of the eye or into the brain of healthy untreated guinea pigs.

Results. When the glioma tissue culture lines (TC 178 or TC 224) were injected into the brains of untreated guinea pigs and mice, no growth resulted.

In contrast, 3 types of lesions were found in the subcutaneous tissue of treated hamsters.

1. Small aggregates of injected cells, the majority of which were necrotic; there was no evidence to suggest that growth of these had occurred after transfer.

2. Nodules ranging up to 7 mm in diameter were found in the majority of animals. These were composed of a necrotic center surrounded by tumor cells growing in clusters without apparent organization.

3. In rare instances larger growths up to 1 cm in diameter were found; unlike the other lesions obtained, these lesions were vascularized and obviously represented true neoplasms of the transplanted cells. It was of particular interest that these tumors had an epithelial-like appearance (Fig. 1) and did not bear any morphological resemblance to human glioblastomas. To supply adequate material for investigation, a further attempt was made to transplant these interesting neoplasms to guinea pigs.

The anterior chambers and brains of untreated guinea pigs were again used as transplantation sites for growing tumor transplants from the treated hamsters. In contrast to the original transfers from tissue culture, these transplants survived and grew. Growth was rapid and within 1½ months large neoplasms were present in both brains and eyes (Fig. 2). The tumors were readily transferred to other untreated guinea pigs and have been carried by serial passage to the present time.

The histological appearance was maintained and became more apparent in the guinea pig transplants. The cells were unquestionably epithelial in character and the tumor appeared to be a carcinoma rather

† Available from Microbiological Associates, Washington, D. C.

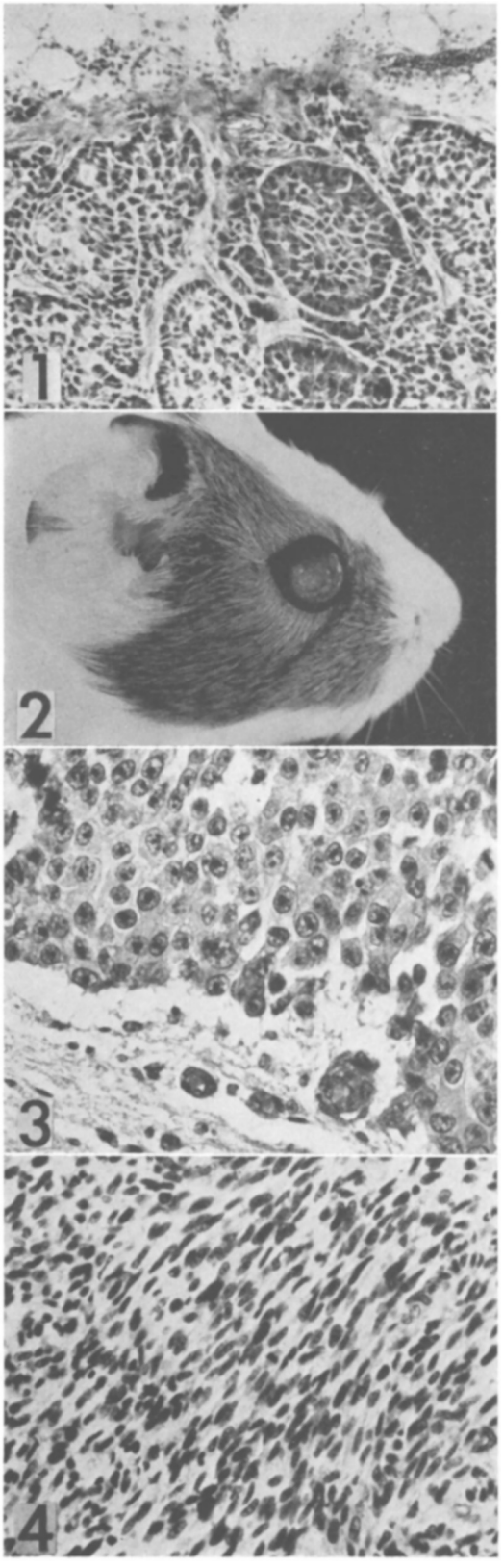

FIG. 1. Epithelial-like tumor produced by glioma TC 224 cells growing in subcut. tissue of treated hamsters. Hematoxylin and eosin stain. × 264.

FIG. 2. Well vascularized tumor produced by glioma TC 178 cells occupying anterior chamber of eye of untreated guinea pigs.

FIG. 3. Epithelial tumor produced by glioma TC 178 cells invading brain tissue of unconditioned guinea pig. Tumor has a carcinoma-like appearance. Hematoxylin and eosin stain. × 660.

FIG. 4. Tumor produced after transplantation of human glioblastoma multiforme directly into brain of untreated guinea pig. Tumor is composed of spongioblast-like cells and closely resembles the human neoplasm. Hematoxylin and eosin stain. × 660.

than a glioblastoma (Fig. 3). It is probably significant that the same morphological alteration occurred in transplants of both of the tissue culture lines (TC 178 and TC 224). It has been shown previously(1) that direct transfer of human glioblastoma tissue to the guinea pig without intermediate tissue culture and hamster transfer results in growths of typical glioblastomatous character (Fig. 4).

It is apparent that the morphological alteration occurred in the tissue culture because it was observed both in treated hamsters and untreated guinea pigs. On the other hand, the attainment of the property of heterotransplantability was somehow related to residence in treated hamsters because cells obtained directly from tissue culture were not heterotransplantable.

Summary. Two tissue culture lines of human glioblastoma multiforme (TC 178 and TC 224) growing in continuous cultivation have been successfully transplanted into the anterior chamber of the eye and into the brain of untreated guinea pigs after a preliminary passage through X-rayed hamsters treated with cortisone. Once heterotransplantability is established, the produced tumors can be transferred from untreated host to untreated host. Histologically the tumors are epithelial-like rather than glioblastomatous.

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Inhibition of the Infectivity of Poliovirus Ribonucleic Acid by an Interferon.* (26712)

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The site at which interferon inhibits virus multiplication has been assumed to be an intracellular one, since interferon does not interfere with adsorption or release of virus (1,2). It was thought that further evidence with regard to its site of action could be obtained using infectious ribonucleic acid (RNA). If the infectivity of intact virus but not that of infectious RNA were inhibited in a cell treated with interferon, it could be assumed that interferon acted at the site of penetration of intact virus, or at the site of reactions involved in dissociation or uncoupling of the intact virus into its infectious nucleic acid and protein components. On the other hand, if the infectivity of RNA as well as intact virus were blocked in interferon treated cells, this would be supportive evidence that the site of interference is at some point of the viral reproductive cycle mediated by infectious RNA. If in addition it could be shown that infectious RNA is adequately taken up and is not preferentially inactivated by cells treated with interferon, the thesis that interferon acts at some intracellular site of virus synthesis or assembly would be further supported. These hypotheses were tested in experiments described in the following report.

Materials and methods. Viruses, cultures and media. Sindbis virus (Egypt AR 339), vesicular stomatitis virus (VSV, Indiana), and poliovirus type III (Saukett) were employed. Primary cell cultures of chick embryo, monkey kidney and human amnion were prepared in 3 oz. bottles. These were nourished in medium consisting of 0.5% lactalbumin hydrolysate (LAH) in Hanks' balanced salt solution (BSS), 4% calf serum and antibiotics. Plaquing medium included in addition 1.5% bacto agar, and 1.0% or 1.5% of 1:1000 neutral red depending on whether monkey kidney or chick cultures were overlaid. *RNA.* Infectious poliovirus RNA was extracted by the cold phenol method from partially concentrated poliovirus harvests of infected Roux bottle cultures of monkey kidney or human amnion cells by the modified method of Holland *et al.*(3). The infectivity of RNA was also determined by their method(4) as follows: RNA was diluted 1:1 in 2M $MgSO_4$ and 0.2 ml was inoculated in monkey kidney cultures that had been washed sequentially with 5 ml phosphate buffered saline (PBS) and 1 ml 2M $MgSO_4$. After 15′ adsorption at room temperature, the inoculated cultures were decanted, washed with 5 ml medium and overlaid with plaquing medium. Various lots of RNA titered 10^2-10^3 p.f.u./0.1 ml. Its infectivity was destroyed by incubation for 20′ at 25° with 10^{-2} μg/1.0 ml of ribonuclease (Worthington), or for 5′ at 25° with medium. *Interferon and controls.* An interferon, hereafter abbreviated S-VIF, was prepared as follows: Stock Sindbis virus titering 10^6-10^8 p.f.u./0.1 ml was first inactivated at 56°C for 4 hours. Five-tenths ml of the inactivated material was added to a chick culture and incubated at 36° for 18 hours, after which it was decanted and washed with 5 ml of Hanks' BSS before addition of 5 ml of medium. The culture then received 0.1

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