THE DEVELOPMENT OF THE RAT EYE IN GRAFT

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(*Received* 5 *February* 1946)

(With Plates 8 and 9)

INTRODUCTION

Although many observations have been reported on the development of the embryo amphibian eye when transplanted either into another individual of the same species or into another part of the same animal, experiments on warm-blooded, and particularly on mammalian, material have been much less numerous. Joy (1939) described the differentiation of retina, optic nerve and tapetum nigrum as well as of sclerotic cartilage and choroid tissue from the isolated optic vesicle of 36 hr. chicks transplanted into the coelum of 48 hr. chick hosts and Glees (1941) obtained differentiated retinal, pigment epithelium and lens tissue when the head of a 12-day rabbit embryo was introduced into the pial covering of the cerebral hemisphere of a young rabbit.

In the experiments to be described it was found that when eyes from embryo rats were grafted under the skin of young animals only those parts derived from the mesenchyme were able to survive even a few days whereas good differentiation of all the eye tissues could be obtained if the transplant was made into the rat brain. Such grafts would 'take' even when graft and host belonged to different strains, although in these cases the proportion of failures was higher than when hosts of the same strain were used. An attempt to graft human embryo retina into the rat brain was not very successful.

No 'host reaction' (Loeb, 1930) was observed when transplants were made into the brain, and this finding confirms those of Murphy (1926) and Siebert (1928) that heteroplastic grafts will grow in brain tissue. Murphy believed that a host reaction depends on the presence of a lymphatic system and that the absence of such a system from the brain explains the ease with which grafts of many different tissues can be made into this organ. He also found that embryo tissue showed no defensive reaction to foreign grafts even when these were from a different species, and he explained this result on the same basis since a lymphatic system had not developed in the embryos he used as hosts. That Murphy's views on the all-important part played by the lymphatic system cannot be accepted as they stand is shown by Greene's (1943) success in transplanting alien embryonic tissue into the anterior chamber of the eye and the testicle of adult rabbits and guinea pigs. The host brains used in the present series of experiments were from animals only 2 days old and at this age the rat brain is still not fully developed. Whether the good results obtained were due to the absence of a lymphatic system or to some other property of brain or embryo tissue or to some combination of these factors, it appears that young brains provide a particularly good environment for the development of many different types of graft.

METHOD

The eyes to be grafted were taken from 15-day rat embryos. These were removed from the head under aseptic conditions and as much of the surrounding tissue as possible removed. If necessary they could be kept for several hours in sterile saline.

Under ether anaesthesia a central incision was made in the skin of the host from the neck to the level of the eyes. A semi-circular flap was then cut in the skull over the left hemisphere and a piece of underlying brain tissue removed to make room for the graft, which was then introduced into the hole so formed on the blade of a Graefe knife and the skull flap allowed to drop back into position. Care was taken to avoid cutting the median vein. The skin was then closed over the wound which was painted over with 2% collodion solution. This was found to be a more satisfactory method of closing the wound than stitching. The rat was put into an incubator at 37° C. and not returned to its mother until it had recovered from the anaesthetic. The whole operation was, as far as possible, carried out under aseptic conditions.

The host rats were killed at intervals after the operation varying from 24 hr. to 300 days. When the skin was removed the graft could usually be seen as a pigmented spot just beneath the skull. It often came away with the bone when the brain and skull were separated, but sometimes remained behind embedded in the brain tissue. In the former case the graft was cut off the bone before fixation, in the latter it was fixed together with the surrounding brain tissue.

The specimens were fixed in Zenker's solution and serial paraffin sections made which were stained with haematoxylin and eosin or by the azan method.

In some cases the embryo lens only was grafted and in others the eye was lentectomized before the graft was made.

RESULTS

The mortality among the host rats was very low. Death was usually due to failure to recover from the anaesthetic, but occasionally the young rats were killed or neglected by their mother after the operation. Sepsis in the operation wound was rare. Altogether about 500 operations were performed.

In about 70% of the operations foreign tissue was found in the brain on histological examination. The proportion of 'takes' was less the longer the host was allowed to survive the operation, but healthy eye tissue was found as long as 294 days afterwards when graft and host were of the same strain. The longest survival when the graft was made into a different strain was 167 days.

Often the only eye tissues found when the brain was sectioned were fragments of retina and lens, but in some cases the whole eye was present with all its tissues, those of mesodermal as well as those of ectodermal origin, being easily recognizable and well differentiated. Lachrymal gland, conjunctiva and skin with hair follicles were sometimes found lying outside the bulb in the surrounding brain.

Pl. 8, fig. 1, shows the stage of development reached by the eye at the time of grafting. It will be seen that the development of the lens is nearly complete, but that most other parts of the eye are still in a very immature condition. The retina is composed of two layers only, the optic nerve fibres and a thick layer of undifferentiated cells showing many mitoses along the outer edge. There is no iris or ciliary body and the sclera, choroid and cornea are still in a very rudimentary condition.

Pl. 8, fig. 2, is taken from an eye 13 days after grafting and, since the gestation period of the rat is 21–22 days, is, therefore, equivalent to one from a 6–7-day-old rat (fig. 3). Although the relative dimensions of the eyes in figs. 2 and 3 are not the same and there is some disturbance in structural organization in the grafted eye, it is clear that all the essential structures have differentiated in the graft. There is an easily recognizable ciliary body and iris, the sclera and cornea have developed and the retina has reached the same stage of differentiation as in the normal eye. Lachrymal gland tissue has also developed outside the bulb. Figs. 4 and 5 show the normal eye at 14 days and an eye that was allowed to develop for 21 days in graft so that it is of an equivalent age. Retinal development has proceeded at the normal rate in the grafted eye and the rod layer has appeared.

Pl. 9, fig. 6, shows that after an eye is grafted it does not continue its development undisturbed. This eye was removed 2 days after the graft was made and the picture is typical of the condition found in twenty grafts during the first few days after the operation. Practically the whole contents of the posterior part of the bulb are necrotic, the only healthy tissue left being the pigment epithelium, the extreme periphery of the retina and the anterior epithelium of the lens. This widespread degeneration of the more highly developed parts of the interior of the eyeball is probably due to the removal of its normal circulation when the eye is cut out of the embryo head. Under these conditions only those parts which are in fairly close contact with the cut surface of the host brain (e.g. the pigment epithelium) or those which are still at a relatively early stage of development seem able to survive. After 3–4 days the graft becomes vascularized by ingrowth of vessels from the host brain tissue.

DISCUSSION

It seems that one must assume that the lens and retinal tissue found in those eyes surviving for a week or longer as a graft must develop from the fragments remaining after the degeneration depicted in Pl. 9, fig. 6, has taken its course. The new retinal tissue presumably derived from the surviving ciliary region and the lens fibres from the lens epithelium. No direct evidence was obtained that new retinal tissue is developed from the surviving peripheral ring but, in the absence of indications of another source, this seems the most reasonable assumption. At every stage of development the peripheral part of the retina is less mature than the central (cf. Pl. 8, figs. 3, 4) and mitosis is particularly active in this region. Further, in fig. 4 the increase in amount of retinal tissue and lack of a properly organized structure, shown in the appearance of rosettes in the periphery, suggests that this was a site of rather active growth. Both Ask (1926) and Stone (Stone & Zaur, 1940; Stone & Chace, 1941) reported degeneration of the central retina followed by regeneration in transplanted salamander eyes. The latter states that this regeneration originates in the ciliary region of the retina.

There is no doubt that the production of new lens fibres is due to the activity of the surviving lens epithelium. In several grafts pieces of epithelium became detached and formed fibres on their own away from the main body of the lens, while in others a lens, which appeared on superficial examination to have developed normally, was found to have more than one equator. Pl. 9, fig. 7, shows such a lens. It is from an eye which was allowed to develop in graft for 21 days and is, therefore, equivalent to a normal 14-day lens. By this age the rat lens is fully developed and the anterior part of the one illustrated in fig. 7 is perfectly normal. The epithelium is reduced to a single layer of cells and the equator (*AB*) is easily recognizable. There is, however, another small piece of epithelium at the posterior pole which has produced its own lens fibres, so forming a second equator at *CD*.

The production of lens fibres from isolated pieces of lens epithelium has already been described by

several workers. Stone & Sapir (1940) found that a new lens could be produced in lentectomized eyes of anuran, urodele and fish larvae by the implantation of lens fragments so long as these included the equatorial region. They show photographs of multilobed lenses very like that in Pl. 9, fig. 7. They were unable to obtain Wolffian regeneration of the lens in these species. van Deth (1940) described differentiation of lens fibres from epithelium in the embryo chick and Fleisher (1921) claimed that the epithelial cells in his auto- and homografts of guinea-pig lens attempted to regenerate fibres. Riedl (1939) found small free 'lentoids' in eight patients belonging to a family suffering from hereditary perinuclear cataract. In all these individuals the lens had been removed at an early age and Riedl suggests that the lentoids, which were composed of true lens fibres, had developed from fragments of epithelium left behind at the time of the operation.

This regeneration of lens fibres from the epithelial cells cannot be dependent on the presence of other ocular tissue since it occurs when lens epithelium only is transplanted (van Deth) or where the whole lens is removed and grafted. The latter experiment was performed several times by the method described above and in nearly every case one or more multilobed lenses were recovered later. The production of such lenses was taken as indicating that the original fibres had degenerated and been replaced by new ones developed from the surviving epithelial cells.

An attempt was made to discover whether Wolffian regeneration of the lens from the iris epithelium under the influence of the retina (Wolff, 1894) can occur in the rat. In these experiments the lentectomized eye was used for the graft. Although lens tissue was undoubtedly produced in several cases it was impossible to be absolutely certain that no lens epithelium was introduced in the graft. It is extremely difficult to remove the lens from an embryo eye without leaving fragments of its epithelium behind, an experimental complication which has not always been sufficiently recognized in past work on Wolffian regeneration.

The induction of lens tissue from iris epithelium by the retina, first described in Amphibia by Wolff, has been shown by Stone and his colleagues to occur in the salamander (Stone & Chace, 1941; Stone, 1943) but not in some other species of Amphibia or in fish (Stone & Sapir, 1940). Positive results were also obtained by Mouroy (1939), Zalokar (1941) and Dinnean (1942), while Alexander (1942), like Stone, could obtain no regeneration in fish embryo eyes. This type of lens regeneration has also been described in the chick by Barfurth (1906) and, more recently, by van Deth (1940), but does not appear to have been observed in mammals.

SUMMARY

1. Successful grafts were obtained when 15-day embryo rat eyes were transplanted into the brains of 2-day old rats.

2. In the grafts differentiation of the various ocular tissues proceeded at the normal rate.

3. The lens and retina first degenerate and then regenerate, the lens from its epithelium, the retina from its ciliary area.

4. It was not possible to establish whether or not Wolffian regeneration of the lens can occur in the rat.

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EXPLANATION OF PLATES

PLATE 8

Fig. 1. Eye of normal 15-day embryo rat. Zenker. Haematoxylin and eosin. ×64.

Fig. 2. Eye of 15-day embryo rat 13 days after being grafted into the brain of a 2-day-old rat. Equivalent age: 6–7 days. Compare fig. 3. Zenker. Haematoxylin and eosin. ×56.

Fig. 3. Eye of normal 6-day-old rat. Zenker. Haematoxylin and eosin. ×16.

Fig. 4. Eye of normal 14-day-old rat. Zenker. Haematoxylin and eosin. ×16.

PLATE 9

Fig. 5. Eye of 15-day embryo rat 21 days after being grafted into the brain of a 2-day-old rat. Equivalent age: 14 days. Compare fig. 4. Zenker. Haematoxylin and eosin. ×12.

Fig. 6. Eye of 15-day embryo rat 2 days after being grafted into the brain of a 2-day-old rat. All the ocular tissues except the pigment epithelium, lens epithelium and extreme periphery of the retina are necrotic. Zenker. Haematoxylin and eosin. ×56.

Fig. 7. Lens of 15-day embryo rat 21 days after being grafted into the brain of a 2-day-old rat. Note the two equators *AB* and *CD*. Zenker. Haematoxylin and eosin. ×32.

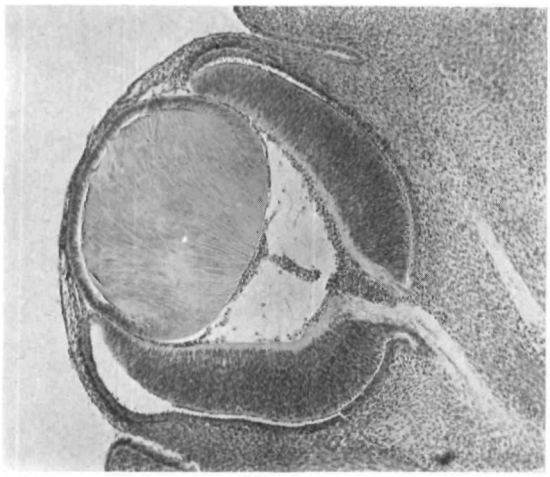

Fig. 1

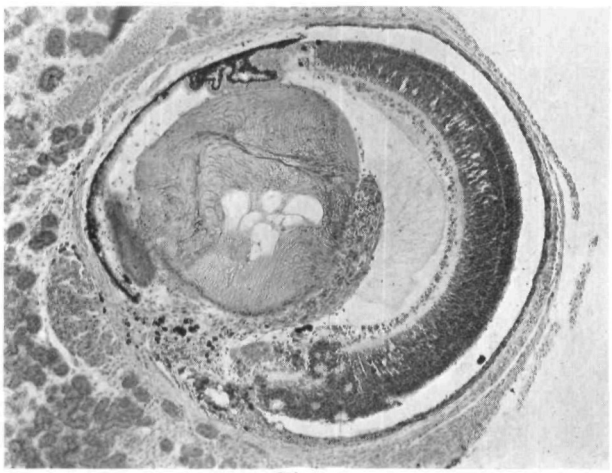

Fig. 2

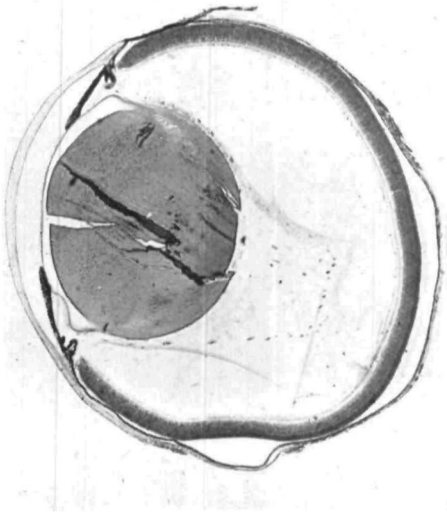

Fig. 3

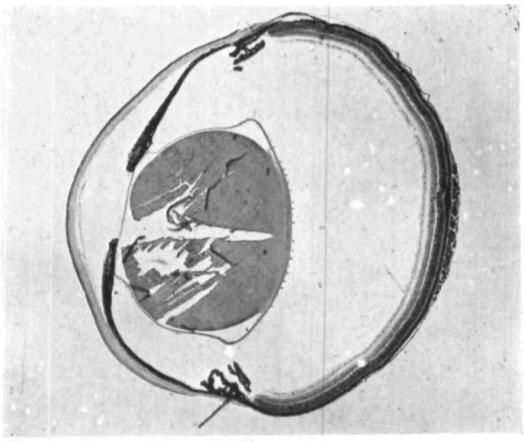

Fig. 4

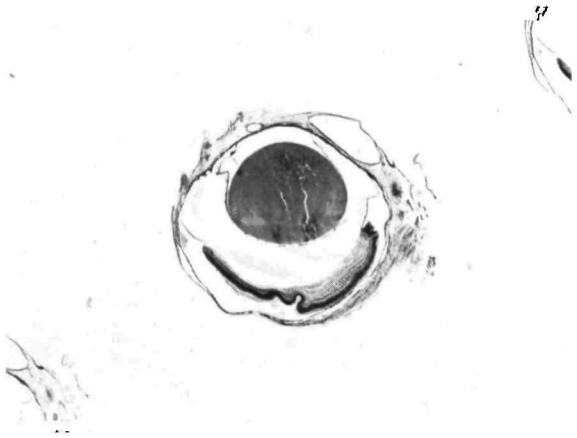

Fig. 5

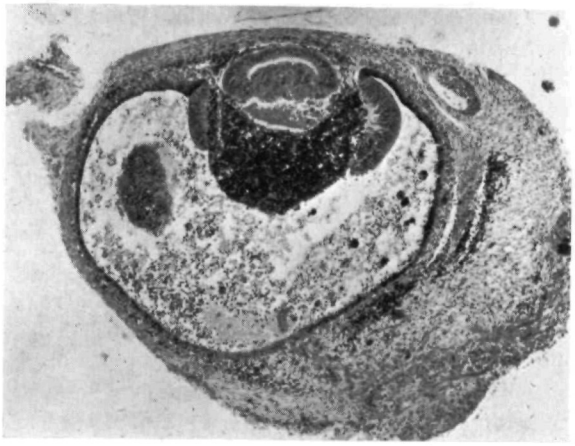

Fig. 6

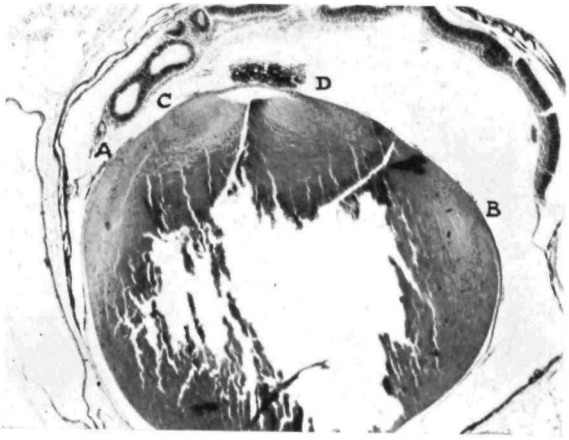

Fig. 7