Growth of Embryonic Gut and Stomach on the Exterior Chest Wall of Adult Cortisone-treated Homologous Hosts

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During the course of recent experiments on skin homografting, rabbit embryos of various ages and mixed stock were employed as donors and adult rabbits, also of mixed origin, as recipients. The youngest embryos used were 13 days post-coitus (normal gestation is 32 days). They were so small (.7–1.0 cm.) that it was very difficult to separate the transparent skin as such, and it was suspected at the time that several layers of tissue and even organs were included in the transplant. Two separate experiments with ten adult recipients were done with embryos of this size. Seven of the hosts received cortisone, according to a technic previously reported (2–4) for successful heterografting, and three were untreated controls. Sections of the grafts taken at 30, 45, and 60 days following grafting showed that epidermis and cartilage were present in all the grafts, as will be reported in detail in another paper. In four of the cortisone-treated hosts, there were small, circumscribed, moist and red but not raw-looking areas on the grafts. Histological examination of such regions proved them to be healthy gut with the crypts opening on the chest wall surface. All these areas gradually increased in size, always being sharply demarcated from the surrounding epithelium. On the 83rd day, when the rabbits were sacrificed, the largest of the “gut” regions was 1 cm. in diameter. They were in good condition both morphologically and histologically (Fig. 4).

These preliminary findings indicated that a method was available for growing the secreting surface of gut, exposed to the exterior and thus easily accessible for experimental study. A series of experiments was therefore undertaken in order that the results described, which had been entirely incidental to another experiment, might be confirmed, if possible, in more detail. Both rabbit and rat embryo gut were grafted on their homologous hosts, and a few rabbit embryo stomachs were also tested on adult rabbits, as will be described.

MATERIALS AND METHODS

Thirty-three young adult female rabbits of mixed or of New Zealand White stock, weighing 3.0–4.0 kg., were used as host animals. Twelve pregnant does of similar origin supplied the embryos which were of four age groups: 14–16, 18–20, 23–25, and 29–31 days. There were three mothers for each set. Three rabbits plus the mother were grafted with gut from the embryos of any one pregnant doe except in the youngest group, where so little material was available that only two rabbits plus the mother were grafted; in this series, also because of paucity of material, skin from older embryos was used to fill up the graft bed. In each group one rabbit, not the mother, was an untreated control; the remainder received cortisone. There were thus, in the total of 45 rabbits, twelve controls and 33 conditioned hosts. All these rabbits were anesthetized with paraldehyde injected intramuscularly (approximately 3 ml. in each hind leg). In the mothers, the gravid uteri were removed intact, and the abdomen was closed with sterile catgut before they were used as hosts for grafts from their own or other embryos. Graft beds were prepared on the chest wall in the manner described in detail by Medawar (1). An area approximately 10 by 7 cm. was marked off with a scalpel knife, and the demarcated epidermis and dermis were stripped off with the same sharp blade. The muscularis, with its overlying fascia and vessels, was thus exposed, and on this richly vascular bed the grafts were placed. Gut for grafting was prepared in one of two

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ways. After removal from the embryo it was either sectioned in strips appropriate to the dimensions of the graft bed or chopped into small pieces with a scalpel. The strips were split open down their whole length and laid down with the exterior surface adjacent to the host fascia. Minced material was merely spread on top of the fascia. Usually a combination of strips and mince was employed plus one or more pieces of embryo skin as control tissue. In the youngest age group, for which a dissecting microscope was necessary to dissect out the gut, no strips were obtained which could be split lengthwise, so that most of the material was graft-ed as a mince; a few pieces were placed on the bed whole. In the next youngest group, minced or whole pieces were also commonly used, though a few split strips were employed. Embryo stomach as well as gut was grafted on the rabbits of one of the 23–25-day sets and one of the 29–31-day groups. Since each of these was composed of a mother and three additional rabbits, there was a total of eight rabbits, six conditioned and two controls, on which this organ was placed. The stomach had first been slit open, then flattened by means of snips with scissors along the edges, and finally wiped dry with sterile gauze. It was laid on the graft bed with the outer surface adjacent to the fascia; the inner surface faced upward. All grafts were dusted with sulfadiazine powder and covered in turn with vaseline gauze, a transparent bandage, and a gauze pressure pad before being wrapped with rolled gauze and adhesive tape. First examination was not made until the 12–14th postoperative day, since prior unwrapping would cause too much stripping and bleeding. Too great a delay, on the other hand, was likely to produce drying and adhesion of the bandages. Great care was taken to create as little trauma as possible to the grafts and the hosts. For this reason a horizontal slit was made in the wrapping, and only the inner pads were removed when the animals were examined. The graft beds were then redusted with sulfadiazine, and new vaseline gauze, transparent bandage, and pressure pads were applied and the whole lightly rewrapped with tape. Examination was subsequently done once a week. By the 4th week, only a light layer of vaseline gauze separated the graft and tape wrapping. After 5–6 weeks, all bandages could be removed if the rabbit was a docile one and not likely to rub itself against the cage or bite at the graft. However, the general practice was to retain a light bandaging, since the grafts, in general, thus remained in better condition.

Eighteen young Wistar female rats, weighing approximately 100–120 gm., were also used as hosts in these experiments, and seven pregnant mothers of the same strain supplied three age series of embryos from 15 days old to almost term (gestation period is 21 days), and, in addition, were grafted. There were three mothers plus two host rats for the embryos of each in the 15–14-day group and two mothers plus three host animals for each in the 16–17-day and also in the 20-day-old series. Again one rat in each group, not the mother, was a control animal, and the remainder were conditioned with x-radiation plus cortisone—a total of seven untreated and eighteen treated hosts. Only embryo gut was grafted; no stomach was used. Anesthesia was nembutal (1.1–15 ml intraperitoneally/120-gm rat). Preparations of graft beds and wrappings were similar to those used for the rabbits but on a smaller proportional scale. Total wrapping, however, could often be removed by the 4th week after which, if the rats were kept in individual cages, the grafts were freely exposed. The rabbits which received cortisone (Cortone, Merck) were given subcutaneous injections at the time of grafting and daily thereafter except Sundays. Animals weighing 3–3.5 kg. were given an initial dose of 30 mg. and a daily dose of 7–8 mg. Larger rabbits of 3.5–4. kg. received a primary injection of 35 mg. and subsequently 5–10 mg. daily. Rats, depending on their size, were given 150 r or 200 r total-body x-radiation 1–4 days prior to grafting plus 3 mg. of cortisone at the time of grafting and 3 mg. twice a week thereafter. Prior conditioning of either rabbits or rats with cortisone was not desirable, as will be noted elsewhere.

All the control hosts were in good condition throughout the experiment. The conditioned rats also remained in good health without the use of antibiotics, but approximately a fourth of the cortisone-treated rabbits developed mild to serious symptoms of pneumonia due to Pneumococcus and/or Proteus vulgaris; these organisms were controlled with penicillin and chloromycetin. One rabbit died from anesthetic in the 18-day group, and one in the 23–25-day group broke its leg and was destroyed 3 days postoperatively. None of the grafts became infected. Two or three of the conditioned animals, depending on the number available, were killed from each embryo-age set, either rabbit or rat, at 1, 2, and 3 months postoperatively. The whole graft was usually photographed at this time and then cut into strips, labeled according to area, for histological sections. Approximately half of these specimens were cut serially. No biopsies were taken, in order that the graft might remain intact and without distortion due to repair processes.

RESULTS

The experimental animals were divided into groups on the basis of age of embryo used, because previous experience had shown that skin and cartilage from very young embryos could often be maintained in an untreated normal host. This finding did not hold true for the gut in the experiments to be described. Although the youngest material did grow the most vigorously, as estimated by relative “spread” of grafted material, gut from any age embryo proliferated on all the cortisone-treated hosts, whether rabbit or rat, and failed to “take” on untreated control animals. Likewise in the limited group of eight rabbits where embryo stomach grafts were used, growth occurred on the six conditioned hosts only.

At the first dressing of the grafts, approximately
14 days after the operation, there was a complete slough in all the untreated control hosts. In the conditioned animals the young gut and stomach grafts derived from any age embryo were very red and prone to bleed owing to the trauma incurred in removing bandages. In contrast, the embryo skin, when present, was much less "friable" and appeared quite pale and smooth. Because of the difference in the two types of surface represented, this finding was not unexpected. At the time of the second dressing, 21–25 days after the grafting, the gut and stomach were no longer "raw." In the rabbits, gut grafts from the younger age groups had by then assumed the appearance which they subsequently maintained, illustrated in Figure 1. Here, 60 days after grafting, are seen large, corrugated, red, moist areas that originated from a mince of 18-day rabbit embryo gut. They are interspersed with hair-bearing nodules formed by the embryo skin which was used to fill out the graft area. In grafts from older rabbit embryos where gut strips were used, the surface was more likely to be smooth (Fig. 2). If mince material was employed, it again produced a nodular growth (Fig. 2, bottom especially the lower right). The strips and also the minced material from the older age groups did not grow as rapidly as the mince from the more immature embryos. Other than this there was little difference in the final results obtained from the different donors. It is noteworthy that the nodular areas derived from minced material were more likely, as time went on, to remain sharply demarcated from the surrounding host epithelium than the grafts from "strip" pieces. When the last eleven rabbits were killed 90 days after grafting, four of five which had received minced tissue still had good, well demarcated grafts throughout the experimental period. Of the six remaining, two had been grafted with embryo stomach as well as gut and which were left at 90 days were among those that had retained a healthy, well demarcated graft throughout the experimental period. Of the two rabbits carrying stomach and killed at 60 days, the stomach was slightly overgrown in one but in beautiful condition in the other (Fig. 3A).

The early appearance of the rat gut grafts was quite similar to those on the rabbits. Eventually, however, about half the rat grafts developed an essential difference. Since the rat embryos were very tiny, more mince of the gut was used than strips. As a result most of the grafts were at first very nodular. The graft beds were also quite small so that the surrounding epithelium often contracted and pushed the gut tissue upward. As the latter continued to proliferate, they assumed the peculiar spherical appearance seen in Figure 3. These grafts were healthy, pink (less red than those on the rabbits), and moist. Strangely, the rats tolerated these relatively large, wet, secreting humps very well, even when they were left unbandaged. In general, neither they nor the rat grafts which remained flat became covered with epidermis. None of the six rats killed at 60 days and only one of six rats killed at 90 days showed an overgrowth of host tissue.

Microscopically, all the grafts from either rabbits or rats killed at 30, 60, or 90 days postoperatively showed adult-type intestine or stomach. It was not possible to judge from a slide the age of the embryofrom which the graft had been derived. Since no attempt had been made to put all proximal or all distal embryo gut on any one host, almost any type of intestine could be found in a graft, especially when a mince had been used. Some of the minced material was encysted and buried below other graft pieces, as could be expected since it was not possible to orient such individual pieces at the time of grafting. Indeed it was surprising that most of the mince did grow with the glandular surface flat and facing outward (Fig. 5). The gut strips, of course, as well as stomach, were properly oriented from the first.

Figures 6 and 7 illustrate the microscopic findings on a rat graft, 60 days postoperative, where some overgrowth of epidermis had occurred. A layer of host connective tissue (Fig. 6) separates the two tissues. This is a typical picture. Epithelium and gut were rarely, if ever, adjacent. The graft itself is in excellent condition and full of mitotic figures (Fig. 8). In many regions (not pictured) it was without host covering tissue and therefore still open to the surface. There were also some areas in this graft where many secretory-type cells were present. Such highly active gut, in other hosts, is illustrated in Fig. 9–11; the first two pictures are from sections of the rat graft seen in Figure 3. Most of the rat grafts and also many of the areas derived from embryo mince grafted on rabbits (Fig. 1) were of this type. The reason for this is unknown. It may possibly account for the fact that there was so little overgrowth of these grafts with epithelial tissues. The secretion produced may have been an inhibitory factor.
Of special interest was the finding that the embryo graft apparently took "in toto," i.e., that not only were the surface cells and glands present, but different layers of underlying smooth muscle as well. In many instances even nerve plexuses (Fig. 12) could be identified. Again the latter were especially common in the rat grafts associated with highly secretory-type gut.

Stomach grafts also showed this "in toto" type of growth, because all the components of the various areas of the adult organ could be identified microscopically (Figs. 13–15). Chief, parietal, and mucous neck cells were present in their usual pattern and relative normal proportions (Figs. 16 and 17). It should be noted that all the stomach grafts had some mucous deposit on their surface. In some instances, the amount produced was considerable.

**SUMMARY**

Embryonic rabbit and rat gut can be grafted successfully on the exterior chest wall of their respective adult homologous hosts if the latter are conditioned with cortisone and, in the case of the rats, x-radiation as well. Limited work with rabbit embryo stomach, grafted on adult conditioned rabbits, has yielded a similar finding. The embryo tissues assume an adult type of morphology, both to the naked eye and microscopically. Grafts have been maintained in a healthy condition as long as 90 days, at which time the experiment was terminated.

**REFERENCES**


**FIGS. 1—Gut and skin graft from an 18-day rabbit embryo, 60 days after being grafted on the chest wall of a cortisone-treated adult rabbit. The gut, which is red, moist, and healthy, is sharply demarcated from both the adult epidermis and the adjacent embryo skin. Hair growing on the latter "bump-like" areas is covered with caked sulfadiazine powder which was used in dressing the graft; it therefore has an odd appearance. None of the powder has remained on the gut, some of which (to the right) is under the rabbit.**

**FIG. 2—Gut from a 24-day rabbit embryo, 60 days after being grafted on the chest wall of a cortisone-treated adult rabbit. This gut was put on in strips except for the lower edge of the graft which originally consisted of minced tissue. Some host epidermis has grown in between the gut, but the latter is more confluent than the picture would indicate.**

**FIG. 3—Gut from a 16-day rat embryo, 60 days after being grafted on the chest wall of a conditioned weanling rat. Although forced to grow upward in the form of a sphere by contraction of the surrounding host epidermis, this gut remained healthy and continued to secrete a slightly viscous fluid. Figures 9 and 10 show some of the histology of this graft.**
FIG. 3A.—Gut and stomach from a 25-day rabbit embryo, 60 days after they were grafted on the chest wall of an adult cortisone-treated rabbit. The gut was put on as a minec and comprises the right lower quadrant of the graft. The rest of the graft is stomach.
Fig. 4.—Gut which grew out from a mince of a 13-day rabbit embryo, 83 days after this mince was grafted on the chest wall of a cortisone-treated adult rabbit. ×40.

Fig. 5.—Gut from a 16-day rat embryo, 30 days after being grafted as a mince on the chest wall of a young adult rat conditioned with x-radiation and cortisone. Numerous mitoses and secretory cells are present. ×75.

Figs. 6 and 7.—Gut from a 16-day rat embryo, same series as Figure 5, 60 days after being grafted on the chest wall of a conditioned young adult rat. Invagination has occurred and epithelium (Fig. 6, arrow) has closed over the vigorously growing intestine which here has the appearance of duodenum or jejunum. Figure 6 is from the region nearest the epithelium, Figure 7 from the base of the graft. In the latter, many glands are present. ×75.

Fig. 8.—High-power view of area near that of Figure 7. Arrows point to mitotic figures. A striated border is present. ×400.
Fig. 9.—Gut from 16-day rat embryo, 60 days after being grafted on the chest wall of a conditioned weanling rat (Fig. 8). This graft was constantly covered with a moist and slightly viscous fluid. As this section shows, it had a highly active, secretory-type surface. It resembles a more distal region of adult intestine than Figures 5-8. X75.

Fig. 10.—High-power view of area marked by arrow in Figure 9. X400.

Fig. 11.—Gut from 17-day rabbit embryo, 60 days after being grafted on the chest wall of a cortisone-treated adult rabbit host. It is very similar to Figures 9 and 10. X400.

Fig. 12.—A plexus of ganglion cells (arrows) in a graft of 16-day embryonic rat gut, 30 days after it had been placed on the chest wall of a young adult rat conditioned by x-radiation and cortisone. In this graft, many other plexuses lay just below glands similar to those of Figure 7 and just above an area of smooth muscle. X400.
Fig. 13.—Stomach from a 30-day rabbit embryo after 60 days on the chest wall of an adult cortisone-treated rabbit. The surface area is to the left. Considerable mucus is being secreted. ×75.

Fig. 14.—High-power view of region indicated by arrow in Figure 13. ×180.

Fig. 15.—Another view of same section as Figures 13 and 14. ×75.

Fig. 16.—High-power view of glands shown in Figure 15. Two parietal cells, among the predominant zymogenic cells, are indicated by arrows. ×750.

Fig. 17.—Another high-power view near Figure 16. Two parietal cells are again indicated by arrows. ×750.
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