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EXPERIMENTAL STUDIES OF EMBRYONIC BONE TRANSPLANTATION

By

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Since *Bert* nearly one hundred years ago (1863) first suggested that embryonic tissues often could be grafted successfully to animals of the same species, much work has been done on embryonic tissue transplantation. An extensive list of references of the early investigations can be found in the monograph by *Bosaeus* (1926).

The variable results of such studies were succinctly summarized by the statement that embryonic tissues may survive homogenous transplantation in whole or in part, permanently, temporarily or not at all (*Willis* 1935).

A review of the accessible literature at the outset of this investigation disclosed no previous work specifically on the subject of embryonic bone transplantation and on its possible use as homogenous bone graft material. It has been stated that the mild reaction to homoimplanted embryonic tissue has led to exaggerated statements and great expectations, but that no greater hope of success can be expected from the homotransplantation of embryonic than adult tissues (*Dempster* 1957). However, a number of theoretical reasons could be marshalled to justify a special study of the homogenous transplantation of embryonic bone, and the chief ones were a) the relatively low antigenicity of embryonic tissues, b) the maintenance of vigorous osteogenesis of embryonic bone in tissue culture with homogenous and heterogenous media, c) the possibility that the fate of a homotransplant might be partly determined by the site of transplantation and therefore that embryonic bone might fare better in the partly protected bed of the host skeleton, d) that some at least of the transplant might survive the so-called "critical period" of *Woodruff*, e) that even if the embryonic bone was ultimately destroyed and replaced by the host bone, this would not necessarily compromise the usefulness of the embryonic bone provided that it produced faster and better initial healing of a defect in the host bone, and finally f) that the embryonic bone tissue might have a stimulating effect on the osteogenesis of the host by a specific inductive process, or even by a non-specific growth-promoting effect.

The special aims of the present work were to investigate 1) the osteogenetic activity and ultimate fate of chick embryonic bone when transplanted to unnatural beds (the muscles and subcutaneous tissues) and to skeletal defects respectively, of adults of the same species, and 2) to compare the final effect on the healing of a standard defect in the host bone filled with embryonic bone as compared to the healing of a standard control defect in the same animal filled with autogenous bone graft material.

METHODS AND MATERIALS

The recipient host animals: All the transplantation experiments were performed in adult roosters. The selection of this experimental species was chiefly conditioned by the facility of obtaining chick embryonic bone of known age at any time for use as homogenous embryonic bone graft material. In addition roosters are quite easy to handle and to house and they are large enough to allow the study of relatively large bone defects. The contribution to the healing by the host and the graft is easier to assess in such large defects and enough callus is provided to subject the material to many different examinations if desired.

The roosters were all apparently healthy adults and were random selected from different farms. They were housed under laboratory observation in cages containing from two to six animals and they all received the same diet consisting of a standard mixed fodder of barley corn and oatmeal.

The donor embryonic material: The donor embryonic bone was derived in all cases from the tibiae of incubated chick embryos of known age. The bones were prepared in the tissue culture room with sterile precautions. The soft tissues, including the periosteum were meticulously scraped off and the cartilaginous epiphyseal cones extruded. The occasional islands of hypertrophied and degenerated cartilage that remain after this treatment are osteogenetically inactive (*Sevastikoglou* 1958).

The tibiae were divided by a scalpel into two or three bits of approximately equal size. The bone bits were suspended in Tyrode's solution in sterile Petri dishes and kept at room temperature until the host animal had been made ready to receive the graft. The delay varied from a quarter of an hour to about one and a half hours.

There is a great difference in the size of the chick tibiae at different ages of incubation. Only 4 tibiae of 18 days old embryonic bone sufficed to fill a standard defect whereas 40 tibiae of bone of 12 days incubation were required. In some cases in order to destroy the vitality of the embryonic bone before it was transplanted the fragments were heated to 50° C for 20 minutes in Krebs-Ringer's solution (bicarbonate free).

Operative technique: In all animals general ether anaesthesia was employed and the incisions were vertical in the midline over the dorsal aspect of the pelvis. The skin was undercut and retracted to expose an adequate expanse of the sacrum. The periosteum was elevated and pushed aside in the selected areas and circular trephine defects, either two or four in number, were made with a hand driven drill of 7 mm. internal diameter. One or two defects were made on one side of the sacrum and corresponding contralateral defects were made on the opposite side equidistantly from the midline. The defects were made through the full thickness of the sacrum and the bone plugs were removed. One defect was filled with embryonic bone material obtained as described earlier. The opposite defect used in all the cases as a standard control, was filled by autogenous bone graft material. The autogenous bone grafts were obtained from the cores of bone removed with the trephine drill. These plugs were divided with scissors into small bits to give an autogenous material roughly comparable in size and quantity with the embryonic material.

In 12 animals (nos. 6–17 incl.) in which four defects were drilled, the two additional defects were used as supplementary controls. The two extra defects were made as described above and placed equidistantly from the midline. One supplementary defect was left empty to determine whether such a defect might heal without any bone graft material implanted. The other was implanted with preheated embryonic bone to see if previous devitalization made any difference to the fate of the embryonic bone or the healing of the defects.

With some care the operation was easy and almost bloodless. The wounds were sutured in most instances with one layer of silk or wire and no dressings were employed. No pressure nor immobilization was necessary.

The aseptic precautions during the operation were fairly elaborate in the beginning but were less stringent in the later cases. Variations in skin preparation, the use of skin towels, wound suturing in one or more layers, irregularities in sterilizing the instruments etc., were apparently of little consequence and only one animal in the whole series showed gross signs of infection.

In the 27 cases in which the embryonic bone was transplanted to soft tissues (subcutaneous tissue in all 27 animals and additional muscle transplants in 10 of them) this was done through separate incisions well removed from the sacral wounds. The muscle implantations were performed in the pectoral region. The subcutaneous implants were placed in pockets in the subcutaneous tissue well away from the skin wound so as not to be involved in its healing process. In all but four cases the soft tissue transplantations were performed in animals in which a bone transplantation experiment was done at the same time.

The results described in the following pages are based on histological, radiographic and contact microradiographic observations. The histological sections of the decalcified specimens were of 5 μ thickness and stained with haematoxylin and eosin, and Azan. A Machlett O.E.G. 50 tube with tungsten target was used for the contact microradiographs of full-thickness sacral areas including the operative defects. Kodak maximum resolution plates and D 158 developer were employed, and the exposure was 10 kV, 10 mA for 10 minutes.

Altogether 53 animals were operated on according to the principles described above. The distribution of the material is shown in Table 1 and is summarized as follows. Transplantation to the subcutaneous tissue alone was done in 14 animals and in 23 other animals the soft tissue transplantation was combined with the transplantation of embryonic bone also into sacral bone defects in the same animal. In the 27 subcutaneous transplantations bone from 12 day old embryos was used in 17 cases, bones from 14 day embryos in 4 cases, from 16 day embryos in 4 cases and from 18 day embryos in 2 cases. Moreover, in these 27 cases which received subcutaneous transplantations of embryonic bone, 10 of them had the embryonic bone transplanted into the pectoral muscles as well, and in all 27 there were additional transplantations of embryonic bone into sacral bone defects. The ages of the embryonic bone grafted to the muscles were 14 days in 4 cases and 18 days in 2 cases.

Comparison of the healing of defects filled with embryonic homogenous and autogenous bone grafts respectively was attempted in 49 animals. In 19 out of the 49 only two defects were made and no other transplantation procedures were performed and in all of these bone from 12 day old embryos was used. In the remaining 30 of the 49 cases, other transplantations, either soft tissue or other bone defect transplantations, were performed in each animal in addition to the standard 2 bone defect operation. In 18 of the 30 cases 12 day embryonic bone was used and in 12 of the 30 cases 4 sacral holes were drilled. In these 12 animals with the 4 defects, the embryonic bone used as graft material was derived from 12 day embryos in 2 cases, 16 day in 5 cases, and 18 day bone in 5 cases.

RESULTS

All the animals sustained the operative procedure well and there were no immediate post-operative deaths. Only one animal (no. 9) developed a purulent infection probably due to technical errors and excessive operative trauma. Twelve animals died before the time planned for sacrifice (nos. 10, 13, 14, 15, 26, 27, 28, 31, 39, 40, 48, 49). No attempt was made to determine the cause of death. Any consideration of the possibility of so-called secondary disease (in which the host sickens and may die due to antibodies developed by the homograft in response to host antigens), was considered beyond the scope of this study. Animals 26, 28, 40, 48, 49 died 14, 10, 6, 8 and 10 weeks respectively after operation and death did not affect assessment of the results. Regarding the other deaths the healing time was less than the arbitrary six weeks chosen for comparison of the healing of the bone defects. Regarding the soft tissue transplants, animals 14, 15, 27, and 31 had none to be examined. In animals 10 and 13 they had already been recovered for histological examination by reoperation before death of the animals occurred at 4 and 3½ weeks respectively. The soft tissue transplants recovered from animal 39 after its unexpected death, showed clear signs of antemortem devitalization. Therefore, although, the twelve deaths represent a mortality of 20 % in the total series it was possible to include most of the material from these animals in the results.

The remaining animals in the series were sacrified at intervals ranging from 5 days to 16 weeks after operation. In 4 cases (nos. 10, 11, 12, 13) the soft tissue specimens were removed by reoperation with sparing of the animal for later sacrifice and examination of the bone defects.

During the earlier stages after transplantation the histological examinations gave the greatest information. Observations on the soft tissue transplantions are based exclusively on histological studies.

1) *The fate of fresh embryonic bone implanted to muscular and subcutaneous beds.*

In 27 animals embryonic bone was transplanted to the soft tissues, in 17 of them only to a subcutaneous bed and in 10 of them embryonic bone was in addition implanted into the pectoral muscles. The operative areas were examined after varying times from five days to 16 weeks. In 12 cases the observation time was 8 to 12 weeks and in 4 cases it was 12 weeks or longer. The average time lapse was 6 weeks.

The sequence of events and the final fate of the transplanted bone fragments were the same both in transplants to muscular and subcutaneous beds. There were no significant differences between transplanted embryonic bone fragments from embryos of different age of incubation.

Up to the end of the 1st week (animals 23, 24, 36) large areas of the implanted embryonic bone showed typical signs of devitalization such as empty lacunae and distorted and absent nuclei and hyperchromatic bone trabeculae. A microcellular reaction was already present around the implants.

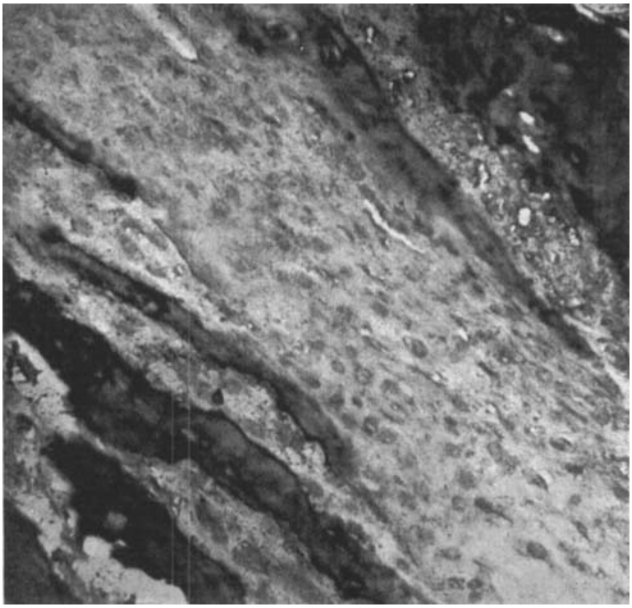


Fig. 1.

Embryonic bone 5 days after transplantation to the subcutaneous tissue, (animal no. 23). Osteoid tissue is visible between the bone trabeculae. There is no microcellular infiltration. Haematoxylin-eosin. Phase-contrast microphotography × 390.

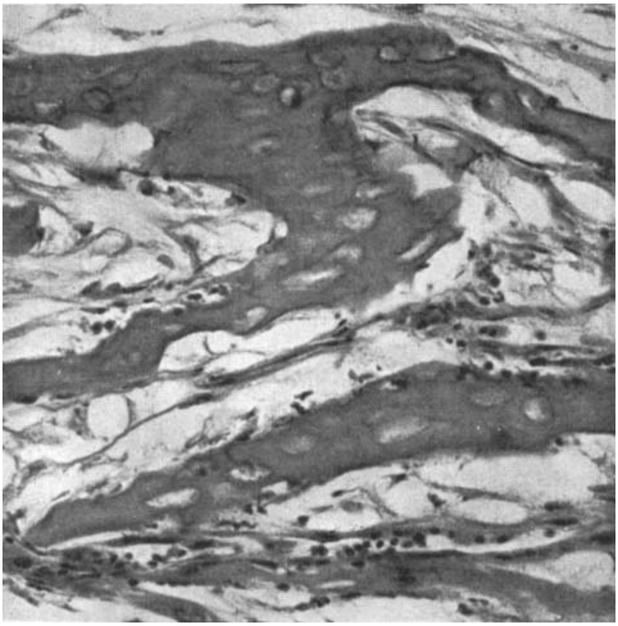


Fig. 2.

Embryonic bone, two weeks after transplantation to the subcutaneous tissue (animal 34). There are apparently viable cells in the uppermost part of the trabeculae at the top of the picture, whereas elsewhere the bone lacunae are empty indicating devitalization. Haemotoxylin-eosin × 390.

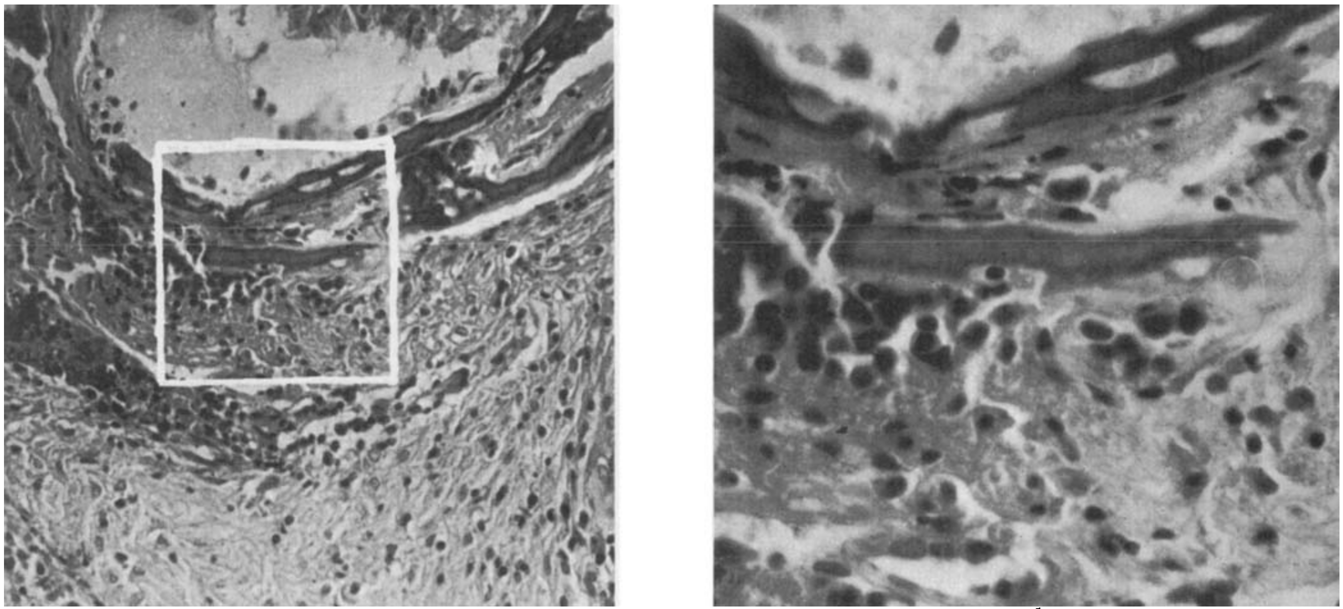
In case no. 23 the transplanted bone was devitalized 5 days after the operation. However, some osteoid tissue could be identified in apposition to the devitalized bone trabeculae (Fig. 1).

At two weeks (animals 34, 35, 39) there was a lymphocytic reaction of varying intensity. The majority of the fragments were clearly devitalized, but in some sections there were still living cells in parts of the trabeculae (Fig. 2).

At three weeks (animals 10, 11, 12, 13, 22, 25) only disorganized eroded trabeculae could be found in the site of implantation (Fig. 3). These debris were strongly hyperchromatic. There was still a prominent small cell reaction, and a fibrous layer isolating the implant began to appear.

In case no. 10, 21 days after the operation, some osteoid tissue was also present in connection with the devitalized trabeculae, limited to a small area.

After that time it was often difficult to find the place of implantation



a *b*

Fig. 3.

Embryonic bone transplanted to the subcutaneous tissue (animal 22) 25 days after the operation. The transplanted bone is completely devitalized. Haematoxylin-eosin *a* × 390, *b* × 975.

either macroscopically or in sectioned specimens. In the majority of cases there were only some traces of scar tissue in the area. In only two animals (nos. 11 and 16) could some remnants of the transplanted bone be found so long as 8 weeks after the operation and in another animal (19) there was some calcified debris within a mass of scar tissue 9 weeks after the operation. The bone remnants were in these cases completely isolated from the surroundings by a well developed fibrous capsule.

In summary, embryonic bone of 12, 14, 16 or 18 days of incubation when transplanted into the muscle or the subcutaneous tissue of homogenous adult animals was soon devitalized. A lymphocytic reaction developed around the transplants. At later stages after transplantation the embryonic bone became completely disorganized and finally absorbed. In the few cases in which some bone remnants could be identified at the site of the transplantation after 4 or more weeks these were isolated by a thick fibrous capsule. No evidence was found of late osteogenesis from the transplanted bone, nor from the host environment by an inductive action of the transplant. In the two cases in which osteoid tissue was identified it was probably produced by the transplanted embryonic bone before its devitalization. The fate of the transplanted bone was the same irrespective of whether one or more operative procedures were performed on the same animal. Thus in the four animals in which embryonic bone was implanted only in the subcutaneous tissue the transplants in animals 23 and 24 were found devitalized 5 days, and in animals 22 and 25, 25 days after the operation. Disappearance of the transplanted bone occurred more rapidly when transplanted to the muscles than when transplanted to the subcutaneous tissue.

2) *Histological comparison of the embryonic bone and the autogenous bone filled trephine defects in the early stages of healing.*

At the end of the first week (animals 1, 36, 37) the defects in the host bone filled with embryonic and autogenous bone material respectively, were found filled with a callus composed principally of fibrous elements, blood capillaries and the transplanted bone fragments. An intense round-cell infiltration was present in the case of embryonic and autogenous transplantations but it seemed to be somewhat more intense around the embryonic implants. The transplanted autogenous bone fragments were in all cases well preserved within the callus mass and the majority of the trabeculae were devitalized. The embryonic fragments were also well preserved within the callus. The young embryonic trabeculae in some areas were still viable but here also the great majority of the implanted trabeculae were dead. In some areas osteoid tissue confirmed by Azan stains was found within the callus in apposition to the embryonic trabeculae and seemed to be arising from them. The autogenous bone fragments were dead and there was no sign of osteogenetic activity surrounding them. Thus it appeared that some of the embryonic bone had preserved its vitality, at this early postoperative stage. Some young bony trabeculae could be found arising from the periosteum of the host bone at the edge of the defect both in cases of embryonic and autogenous implantation.

At the end of the second week following operation (animals 2, 34, 35) the fibrous consistency of the callus was more marked both in embyonic and autogenous transplantations. Almost no round cell reaction was present in the autogenous callus whereas there was still a very intense reaction around the embryonic bone transplants. Both embryonic and autogenous bone was devitalized in all cases and absorption of the transplants was well advanced so that only remnants of both embryonic and autogenous grafts were present within the respective defects. A regeneration of the periosteal layer of the host bone was present in this stage creeping towards and covering the callus on both its surfaces. Bone deposition of periosteal origin was seen on the surfaces of the callus. The development of the periosteal layer and the apposition of the young bone from it was more pronounced in the defects filled with autogenous grafts.

Thus, in this stage of healing there was clear evidence that the embryonic bone had lost its viability. No osteogenetic activity was present within the callus of either the embryonic or the autogenous transplantations and a pronounced absorption of the grafts was observed. Bone healing was arising from the host periosteum and was more advanced in the case of the defects filled with autogenous bone.

By the end of the fourth week after operation (animals 3, 31, 32, 33) most of the transplants had been absorbed and only remnants of both embryonic and autogenous transplants were present within the respective defect and with no sign of osteogenic activity in the calluses. Osteogenesis arising from the host periosteum was very pronounced in the cases of the autogenous bone grafted defects while this was not so marked in the defects filled with embryonic bone.

By this stage the healing process of the defects by contribution from the margins of the defects was much more advanced in the case of autogenous than in embryonic bone transplantation. In both cases there was no appreciable osteogenic activity within the callus. The transplanted embryonic and autogenous bone fragments were devitalized and most of them had already been absorbed.

3) *Comparison of the embryonic and autogenous bone filled defects after 6 weeks or longer.*

In most cases after about four weeks the differences in healing were obvious. Even by gross examination one could assess the relative amount of bone filling the respective defects. The assessment of the amount of bone by gross examination of the appearance, hardness and architecture of the contents of the defects showed good correlation with the amount of bone demonstrated by subsequent contact microradiography. Six weeks was arbitrarily chosen as sufficient time of healing to allow a final comparison of the bone healing process of the respective defects and contact microradiography was used to record objectively the differences in the areal narrowing of the defects by new bone. There were 32 animals fulfilling the time requirement for this comparison (i.e. 6 weeks healing time). In 24 of these 32 animals there was a clear superiority of bony healing of the defects filled with autogenous bone as compared to the bone defects filled with homogeneous chick embryonic bone. In the majority of cases the former defects were completely or nearly obliterated by bone bridging the trephine defects whereas the latter were filled by soft fibrous tissue or a very thin almost transparent connective tissue membrane. The embryonic bone fragments had disappeared and any narrowing of these embryonic bone filled defects consisted of only a little peripheral constriction of the defects by new bone from the host.

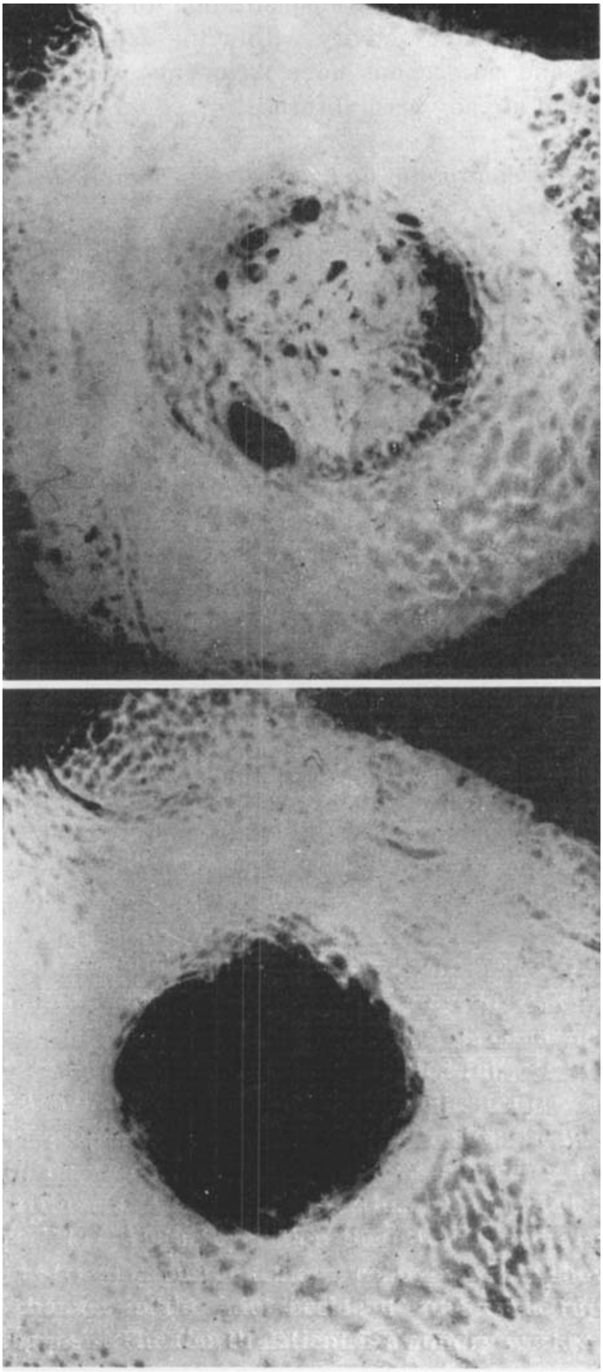
Figures 4, 5, and 6 show examples of the contact microradiographic appearance of bone defects at 8, 10 and 12 weeks respectively.

In only three animals (4, 6, 28) was there a superiority of healing of the defect filled with fresh homogenous embryonic bone, as compared to the control defect filled with autogenous bone.

In four animals (5, 12, 20, 29) there was insufficient difference in the relative healing to record a superiority of one defect over the other.

4) *Observations regarding the animals with 4 bone defects.*

Before it became clear that the embryonic bone was destroyed by the usual homograft reaction, a study was begun employing 4 bone defects as described under methods, and this concerns animals 6–17 inclusive



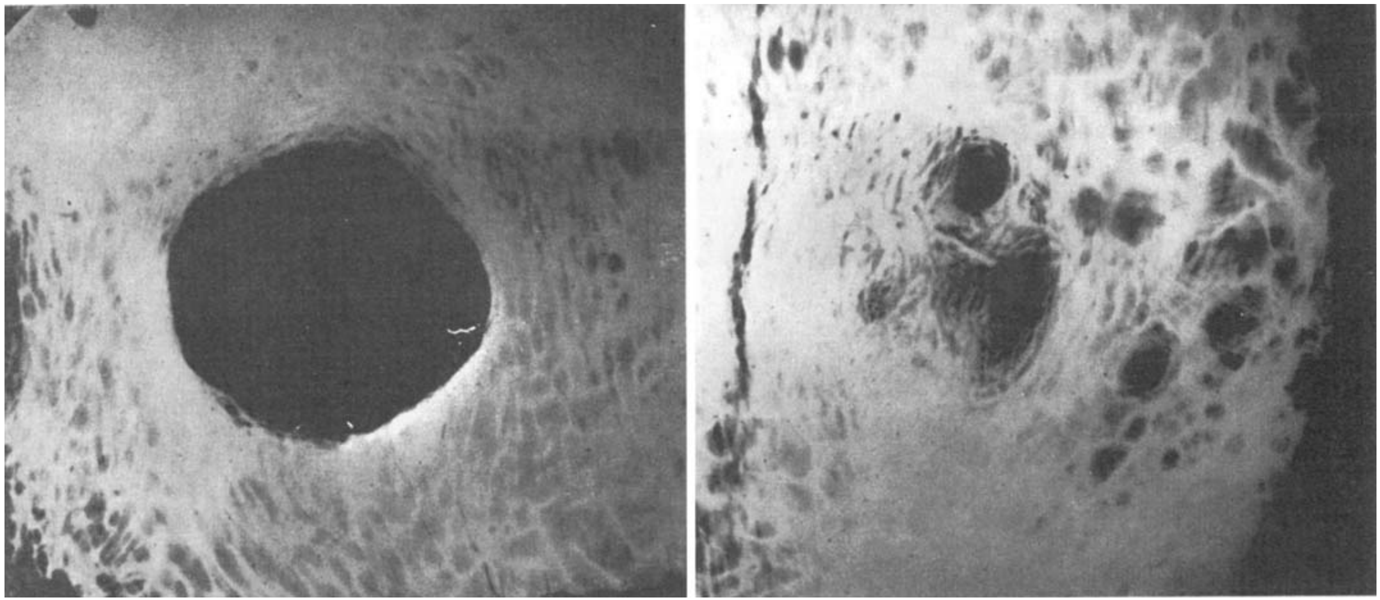
a b

Fig. 4.

Contact microradiographs. Parts of sacrum of rooster no. 53, including the comparative bone defects, 8 weeks after operation.

a) The defect filled with embryonic bone, shows only slight marginal new bone formation.

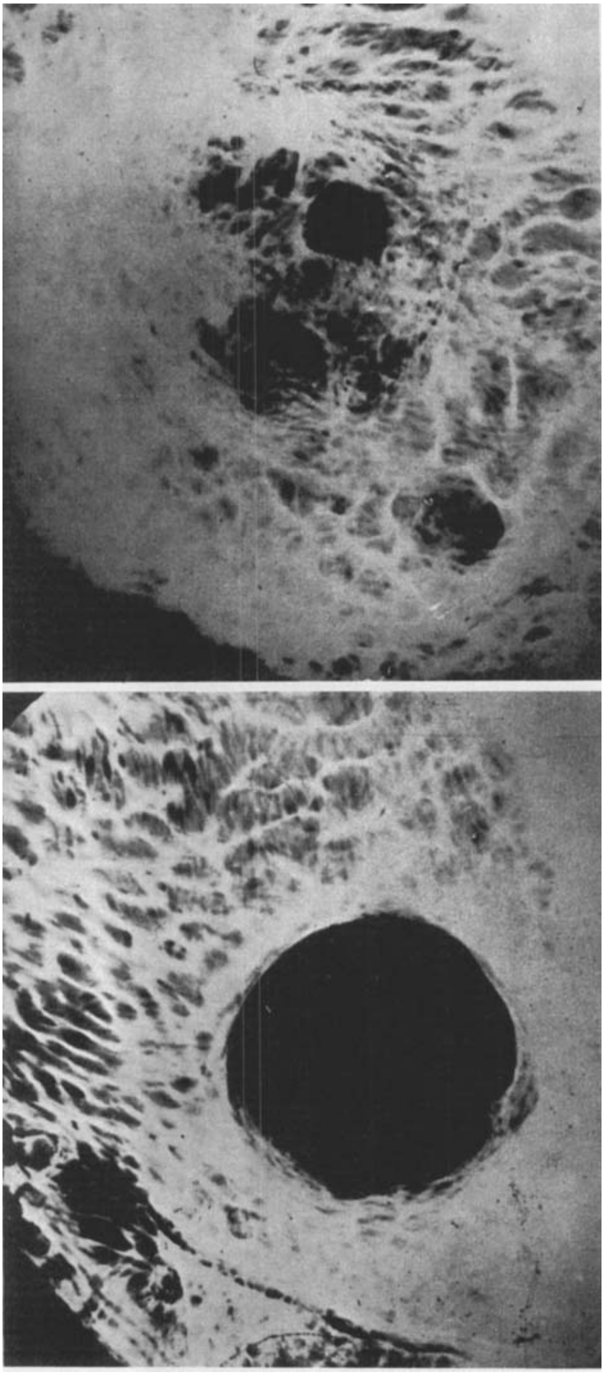
b) The defect filled with autogenous bone chips is almost completely obliterated by bone.



a *b*

Fig. 5.

Contact microradiographs. Parts of sacrum of rooster no. 50, including the comparative bone defect, 10 weeks after operation.
a) The defect filled with chick embryonic bone shows no bony healing.
b) The defect filled with autogenous bone chips is obliterated by bone except for two small eccentrically placed residual defects.



a b

Fig. 6.

Contact microradiographs. Parts of sacrum of rooster no. 42, 12 weeks after operation. a) The defect filled with embryonic bone shows no bony healing. b) The defect filled with autogenous bone is nearly obliterated by bone.

TABLE 1
The Distribution of the Material.

Animal no.	Transplantation						Age of embryonic bone (days)	Time of observation	Comparison of bone in late stages of healing (6 wks. or more)
	Soft tissue		Bony defect filled with						
	Subcut.	muscle	Fresh embr. b. (E)	Auto-genous b. (A)	Heated embr. b. (H)	Empty (O)			
1			×	×			12 d	1 wk.	
2			×	×			12 d	2 wks.	
3			×	×			12 d	4 wks.	
4			×	×			12 d	8 wks.	E > A
5			×	×			12 d	16 wks.	E = A
6			×	×	×	×	12 d	8 wks.	E > A
7			×	×	×	×	12 d	11 wks.	A > E
8			×	×	×	×	18 d	8 wks.	A > E
9			×	×	×	×	16 d	8 wks.	infected
10	×	×	×	×	×	×	16 d	4 wks.	
11	×	×	×	×	×	×	16 d	8 wks.	A > E
12	×	×	×	×	×	×	16 d	8 wks.	A = E
13	×	×	×	×	×	×	16 d	3½ wks.	
14			×	×	×	×	18 d	5 wks.	
15			×	×	×	×	18 d	3 wks.	
16	×	×	×	×	×	×	18 d	8 wks.	A > E
17	×	×	×	×	×	×	18 d	8 wks.	A > E
18	×	×	×	×			14 d	9 wks.	A > E
19	×	×	×	×			14 d	9 wks.	A > E
20	×	×	×	×			14 d	9 wks.	A = E
21	×	×	×	×			14 d	9 wks.	A > E
22	×						12 d	25 days	
23	×						12 d	5 days	
24	×						12 d	5 days	
25	×						12 d	25 days	
26	×		×	×			12 d	14 wks.	A > E
27			×	×			12 d	2 wks.	
28	×		×	×			12 d	10 wks.	E > A
29	×		×	×			12 d	12 wks.	A = E
30	×		×	×			12 d	16 wks.	A > E
31			×	×			12 d	4 wks.	
32			×	×			12 d	4 wks.	
33	×		×	×			12 d	4 wks.	
34	×		×	×			12 d	2 wks.	
35	×		×	×			12 d	2 wks.	
36	×		×	×			12 d	1 wk.	

> = Superior healing.

TABLE 1 (cont.)

Animal no.	Transplantation						Age of embryonic bone (days)	Time of observation	Comparison of bone in late stages of healing (6 wks. or more)
	Soft tissue		Bony defect filled with						
	Subcut.	muscle	Fresh embr. b. (E)	Autogenous b. (A)	Heated embr. b. (H)	Empty (O)			
37			×	×			12 d	1 wk.	
38	×		×	×			12 d	6 wks.	A > E
39	×		×	×			12 d	2 wks.	
40	×		×	×			12 d	6 wks.	A = E
41	×		×	×			12 d	16 wks.	A > E
42			×	×			12 d	12 wks.	A > E
43			×	×			12 d	16 wks.	A > E
44			×	×			12 d	8½ wks.	A > E
45			×	×			12 d	8½ wks.	A > E
46			×	×			12 d	8½ wks.	A > E
47			×	×			12 d	8½ wks.	A > E
48			×	×			12 d	8 wks.	A > E
49			×	×			12 d	10 wks.	A > E
50			×	×			12 d	11 wks.	A > E
51			×	×			12 d	6½ wks.	A > E
52			×	×			12 d	8 wks.	A > E
53	×		×	×			12 d	8 wks.	A > E

(see table 1). This part of the study was then abandoned and only a few general observations can be made.

One bone defect was left empty in all of the cases in order to control the remote possibility that such a defect in the sacrum of a rooster might heal better when left vacant than when implanted with some sort of bone graft material. This showed that a full thickness trephine defect of 7mm. in diameter usually fails to heal. An exceptional case was no. 12 and this animal exhibited such great innate bone healing power that it healed all 4 defects in eight weeks, including the one which was left empty.

The magnitude of the operative intervention, using 4 defects and also soft tissue transplants in the same animal, did not qualitatively affect the general pattern of superior healing of the defects filled with the autogenous bone as compared to those filled with the embryonic bone. The defects with autogenous bone showed better healing than all the other defects.

In these few animals, in which preheated embryonic bone was implanted into one of the defects, the impression was gained that the preheated bone fragments were not disrupted and removed as rapidly as the fresh embryonic fragments. They contributed equally little to the healing of the bone defects as did the fresh embryonic bone fragments. No study was made of animal no. 9 because of infection.

DISCUSSION

The methods: Regarding the part of the investigation dealing with the comparison of healing bone defects, the reasons for placing the control defect in the same animal should be explained.

The study is concerned with the healing of a bone defect by some local application, in this case by homogenous embryonic bone. Generally speaking one can expect any substance used as bone graft material to manifest its superiority locally and not through some central mechanism difficult to control. Thus this is a problem of whether a local treatment of a bone defect produces more or faster bone healing of the defect than an identical control defect in the same animal filled with autogenous bone. Autogenous bone is generally considered as the best currently available bone graft material (*Chase & Herndon* 1955) and it is used as a standard for comparison.

By such a method one can disregard general factors affecting the bone healing of one animal as compared to another, as for example, the age, the diet, the general state of health and the inborn healing capacity. In this connection there was observed a considerable variation in the bone reparative power of these animals. This was particularly obvious in four animals that are recorded as showing insufficient difference between the healing of a defect and the control defect. In one (no. 5) both defects had healed so solidy after 16 weeks that no difference could be discerned. In another (no. 12) which had 4 trephine defects the animal's own bone reparative power was so great that it healed all defects in 8 weeks, even the one that had been left empty. On the other hand, the other two animals (no. 20, 29) showed little or no healing of even the defects implanted with autogenous bone indicating the relatively low healing power of these hosts.

It must, however, be noted that an inherent difficulty in this method of comparison is that it is possible for the superiority of a grafting material to be masked if it does operate through a central systemic mechanism. Such a mechanism might of course accelerate the healing of the control defect too. Germane to this discussion is the work investigating various so-called growth-promoting substances on the healing of skin wounds. For example, *Auerbach & Doljanski* (1944), found that the local application of embryonic and adult tissue extracts did not result in an accelaration of the healing of a treated experimental skin wound as compared to a control wound in the same animal. However, further work using controls in other animals, unmasked a wound accelerating affect that was hidden because it seemed to have operated also on the control wound in the same animal by some general pathway. This led them to investigate the effect of intraperitoneal injection of the tissue extracts on the rate of healing of experimental wounds (*Auerbach & Doljanski* 1945). They concluded that there was a significant accelerating affect agreeing with the results reported earlier by other authors (*Roulet* 1926, *Lorin-Epstein*, 1927, *Amorosi* 1931, *Sandelin & Björkesten* 1932).

These considerations evidently played no significant role in the investigation reported in this communication. If any systemic mechanism was operating either to accelerate or depress the healing of the bone defects, it certainly was not sufficient to obscure the considerable superiority of the autogenous bone which was obvious in the vast majority of cases. This superiority was clear even though the autogenous bone material contained considerable cortical fragments. To be the best possible autogenous graft for comparison the material probably should have consisted wholly of cancellous bone (*Siffert* 1955, *Nicoll* 1956).

The means of assessing the results. The main task of this investigation was to asses the ultimate contribution of the embryonic bone, if any, and by whatever mechanism, to the final healing of the experimental bone defects. This was gauged principally by contact microradiography after a healing time of six weeks or more. The choice of six weeks was purely arbitrary and actually the healing process seemed to be finished in most cases in four weeks and even less. Little difference was observed in the amount of new bone filling the defects from six to sixteen weeks following operation. Apparently the reparative process was exhausted and if a defect had not healed by six weeks there would be little if any further bony healing by sixteen weeks.

In a critique of the methods used to study the rate of healing of experimental wounds, the dangers of observations before the completed stage of healing are emphasized (*Young et al.* 1941). An initial acceleration produced by a substance in an early stage of healing might be nullified by a later retardation or lag-phase in the healing.

Recent work on the acceleration of wound healing by cartilage exemplifies this difficulty (*Paulette & Prudden* 1959). An early acceleration of wound healing was observed but the continued presence of the cartilage in the wound actually delayed the eventual maturation of the scar.

The embryonic bone transplants to the soft tissues and the calluses in the bone defects during the first few weeks of healing were studied by histological methods. These studies were indispensable in establishing the fate of the embryonic bone particularly in the soft tissues but were much less helpful in comparing the healing of the respective bone defects. Sections could be found to support almost any thesis and it is particularly difficult to be absolutely certain of the quantity and origin of the new bone.

The fact that in most cases the soft tissue transplantation experiments and the implantation of the embryonic bone into the bony defects were done together in the same animals was of no significant importance in the fate of the embryonic bone nor on its affect on the healing of the bone defects. Only four animals had soft tissue embryonic bone transplants uncomplicated by bone defect operations at the same time. In these animals the embryonic bone was destroyed in just the same way as it was in the other animals when additional embryonic bone was implanted in the bone defects. Many of the bone defect operations were performed with and without concomitant soft tissue embryonic bone transplantations, and the overall results were the same.

General discussion of the results. It is well known that bones from the rapidly growing chick embryo can maintain their vigorous osteogenetic activity even in tissue culture with homogenous (*Fell* 1931–32) and heterogenous medium (*Miyazaki et al.* 1957). Even the frontal bone of the chick embryo can subsequently show intense growth after having been stored at 4° C for eight days (*Judet* 1954). It is said that certain fetal cells when transplanted can overcome the normal homograft barrier (*Kay & Constandoulakis* 1959) but this evidently does not apply to chick embryonic bone from the twelfth day of incubation and thereafter. This investigation shows that such bone cannot be expected to survive transplantation to adults of the same species. Occasionally early new bone formation was found arising from the transplants and this agrees with other recent work on homogenous bone transplantation (*Chalmers* 1959).

Burke et al. (1944) confirmed earlier work published in the immunological literature by showing that adult organs seem to be more distinct antigenically as well as morphologically. However, *Schlechtman* (1948) and *Ebert* (1950) found organ antigens in the early chick blastoderm and it is possible that adult tissue antigens are present in the earliest stage of development or even in the unfertilized egg and that the concentration merely rises with development (*Tyler* 1955).

There has been a recent revival of interest on embryonic skin grafts partly due to the work of *Toolan* (1958). She showed that homogenous embryonic skin grafts could be maintained indefinitely on rabbits properly conditioned with cortisone but the only grafts retained by unconditioned hosts were derived from very young embryos in the first trimester of gestation. Commenting on Toolan's work *Medawar* (1959) points out that it seems hardly possible that the prolonged survival of the embryonic skin grafts is due merely to the absence of tissue antigens because even if they were absent to begin with they should have made their appearance when the embryonic skin underwent further differentiation on its host. *Goldstein & Baxter* (1958) found evidence of an immunological reaction even around very young embryonic skin homografts in human material. In much earlier work a real and obvious take of embryonic skin was observed for as long as six months after which the embryonic skin disappeared (*Gaillard & Kooreman* 1943). The difficulty of being certain of long term survivals is indicated by *Snyderman* (1958).

Although various experimental work supports the contention that the younger the embryo the less organ antigen is detectable the destruction of the embryonic bone used in this investigation was so outstanding that it is hardly likely that using very young embryonic bone (bone appears in the chick embryo at about the eight day of incubation) would produce results sufficiently different to warrant further investigation along the present lines, unless the host or the graft are modified in some way.

It has been suggested that the site of transplantation has a bearing on the fate of the graft (*Gaillard* 1953, *Greene* 1955). It was hoped that the rapid growth and establishment of the embryonic bone in the environment of the host's skeleton might allow it to withstand an eventual immunological attack. It is alleged that certain homografted tissues which are kept alive beyond a "critical period" are no longer susceptible to the immune response (*Woodruff* 1952). One might hope that the rapid growth of the embryonic bone in an environment perhaps partly protected from access by immunologically active cells could be decisive in enabling some of it to survive this critical period. No evidence of the operation of such adaptive processes could be adduced from the present investigation. Even relatively large amounts of embryonic bone implanted into the sacral defects of roosters were destroyed and removed.

Bone formation by induction is a well known concept (*Levander* 1938, *Urist & McLean* 1953, *Heinen et al.* 1949, *Ham & Harris* 1956, *Moss* 1958) and it needs no elaboration here. Since *Carrel* (1913) demonstrated the potent activating power of extracts of chicken embryos on the growth of connective tissue in tissue culture, the literature is replete with studies of the effect of embryonic and other tissue extracts on the healing of superficial wounds. (*Kiaer* 1927, *Fischer* 1941–42, *Dann et al.* 1941, *Hoffman & Dingwall* 1944 and others). It has been stated that there is almost unanimous agreement that embryonic extracts do accelerate the healing of wounds (*Needham* 1950). In this investigation the defects filled with homogenous embryonic bone and also those filled with autogenous bone material were healed by the ingrowth of the host bone and in almost every case this new bone was less in the defects filled with embryonic bone. When similar defects were left empty (12 animals) there was little or no marginal healing, except in no. 12 which had an extraordinary regenerative capacity and healed the vacant defect as well as three other defects in which graft material had been implanted. Those cases in which implantation of embryonic bone in the defect did result in a little more healing by the host bone than when nothing was implanted, are hardly evidence of any bone growth stimulation or induction, and in any case it is of no clinical importance in the light of the better performance of the autogenous material. Therefore, no significant growth-promoting nor inductive affect on the host bone can be ascribed to the embryonic bone in the bone defects.

Failing any other beneficial action of the embryonic bone one might expect that the smallness and delicacy of the embryonic bone fragments and their low mineral content might allow their more rapid substitution by ingrowth of the host bone than in the case of the autogenous fragments which contained some highly calcified cortical elements. Even small fragments of cortical bone presumably present a greater obstacle to removal and substitution by the host bone. This investigation showed that the bone defects healed principally from the margins. Neither the embryonic nor the autogenous bone fragments contributed any significant osteogenesis. Why then did not the defects filled with embryonic bone heal equally well as those filled with autogenous bone? One explanation may be that the embryonic bone was destroyed too rapidly to act as an efficient scaffold for infiltration of the host bone. One might also consider that the host bone ingrowth was adversely affected by the immunological cellular reaction taking place although *Kelsall & Crabb* (1958) suggest that a lymphocytic reaction should stimulate rather than depress healing.

In the first operated animals, histological and routine X-ray methods seemed to show superior healing taking place in the defects filled with embryonic bone. On this basis the potentialities of embryonic bone as grafting material were overestimated (*Cleland & Sevastikoglou* 1960). The continued investigation reported here has not confirmed these early interpretations.

SUMMARY AND CONCLUSIONS

The fate of embryonic bone on homogenous transplantation and its affect on the inherent normal reparative osteogenesis of the host has been studied by transplantation experiments of chick embryonic bone to 53 roosters.

Tibial bone fragments from chick embryos of twelve days incubation or more, when transplanted to the subcutaneous tissue or the pectoral muscles of roosters, did not survive more than a week or two. In most cases by four weeks the remnants had disappeared leaving little or no trace. Before the destruction of the transplantation material osteoid tissue was seen arising from some of the embryonic bone trabeculae. In no case was any late bone formation induced in the region of these transplantations when examined from two to four months after operation.

When the chick embryonic bone was implanted into full thickness trephine defects in the sacral bones of roosters, some of the embryonic fragments survived at least a week and they even gave rise to some osteoid tissue. Ultimately their fate was the same as with the transplantation to soft tissues without any significant prolongation of survival.

The bone defects filled with autogenous bone fragments healed better than those filled with embryonic bone.

No evidence was adduced of a beneficial effect by the embryonic bone on the reparative power of the host bone.

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RESUME

Le sort d'un os embryonnaire dans une transplantation homogène et son effet sur l'ostéogénèse de réparation normale naturelle chez l'hôte ont été étudiés par des expériences de transplantation d'os embryonnaire de poussin sur 53 coqs.

Des fragments d'os tibial d'embryon de poussin, après 12 jours d'incubation ou plus, ne survivent pas plus d'une semaine ou deux lorsqu'ils sont transplantés dans le tissue sous-cutané des muscles pectoraux des coqs. Dans la plupart des cas, au bout de quatre semaines les restes avaient disparu laissant une faible trace ou pas de trace du tout. Avant la destruction de la transplantation, on a pu voir un tissue ostéoïde se développer de certains des trabécules de l'os embryonnaire. Dans aucun cas il ne s'est produit de formation osseuse tardive dans la région de ces transplantations, examinées entre deux et quatre mois après l'opération.

Lorsque le tissue embryonnaire de poussin a été implanté dans toute l'épaisseur de défectuosités de l'os du sacrum chez les coqs, certains des fragments embryonnaires survécurent au moins une semaine et donnèrent lieu à l'apparition d'un peu de tissu ostéoïde. Finalement leur sort a cependant été le même que dans les transplantations sur tissu mou, sans aucune prolongation de vie notoire.

Les défectuosités osseuses remplies de fragments d'os autogène guérissent mieux que celles remplies d'os embryonnaire.

Il n'apparaît pas que l'os embryonnaire ait un pouvoir réparateur avantageux sur l'os de son hôte.

ZUSAMMENFASSUNG

Das Schicksal von Embryoknochen nach homogener Transplantation und seine Wirkung auf die angeborene normale reparative Osteogenese des Wirtes wurde mittels Transplantationsversuchen von embryonalem Hühnerknochen auf 53 Hähne untersucht. Knochenstückchen der Tibia von Hühnerembrios, die 12 oder mehrere Tage bebrütet worden waren, überlebten nicht mehr als ein oder zwei Wochen, wenn sie in das subkutane Gewebe oder den m. pectoralis von Hähnen verpflanzt wurden. In den meisten Fällen waren die Reste nach 4 Wochen verschwunden unter Hinterlassung von geringen oder keinerlei Spuren. Vor der Zerstörung des Transplantationsmateriales konnte in einigen Fällen osteoides Gewebe, das von den embryonalen Knochentrabekeln ausging, beobachtet werden. In keinen Falle war irgendwelche späte Knochenbildung herforgerufen worden, wenn man zwei bis vier Monate nach der Operation untersuchte.

Wen der embryonale Hühnerknochen in durchgehende Trepanationsdefekte des Kreuzbeines von Hähnen eingepflanzt wurde, überlebten einige der embryonalen Fragmente zumindest eine Woche und es bildete sich von ihnen aus etwas osteoides Gewebe. Aber schliesslich war ihr Schicksal dach dasselbe als jener, die in Weichteilgewebe verpflanzt worden waren, ohne irgendwelche Verlängerug des Überlebens von Bedeutung.

Die Knochendefekte, welche mit autogenem Knochen ausgefüllt wurden, heilten besser als die mit embryonalen Knochen.

Keinerlei Beweis einer günstigen Wirkung des embryonalen Knochens auf die Wiedererstellungskraft des Wirtsknochens konnte nachgewiesen werden.

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