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GROWTH POTENTIAL OF SUBCUTANEOUSLY TRANSPLANTED CRANIAL BASE SYNCHONDROSES OF THE RAT

by

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INTRODUCTION

The synchondroses of the cranial base have been generally regarded as major centers of growth, effecting the dimensional increase of the cranial base (cf. *Baume*, 1968). Some authors have questioned the importance of these cartilages for cranial growth (*Ortiz & Brodie*, 1949; *Koski*, 1960; *Powell & Brodie*, 1963), mainly on circumstantial evidence. The »functional matrix» theory of *Moss* (1960) seems to imply that the cranial base synchondroses are not independent growth centers but sites of growth, governed by stimuli from their immediate environment, mainly from the contents of the brain capsule.

It has been shown that some growth cartilages, e.g., the epiphyseal cartilages, are capable of independent bone-growth-promoting activity even in non-functional environments (*Lacroix*, 1951; *Felts*, 1957; *Koski & Rönningm*, 1966), whereas the condylar cartilage of the mandible, also generally considered to be a major growth center, has been found to lack a comparable capacity (*Koski & Mäkinen*, 1963; *Koski & Rönning*, 1965; *Charlier & Petrovic*, 1967; *Duterloo*, 1967). In view of these differences, and of the differing opinions regarding the significance of the cranial base synchondroses referred to above, it was decided to study the behaviour of transplanted cranial base synchondroses.

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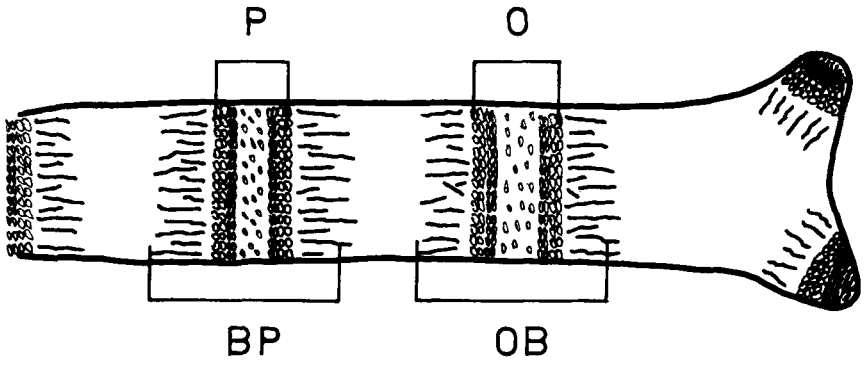


Fig. 1. A schematic drawing of the cranial base depicting the synchondroseal transplant types.

MATERIAL AND METHODS

Non-inbred rats belonging to a Long-Evans stock were used throughout the study. The age of the animals varied from 3 to 7 days, the great majority of them being 5—6 days old.

The following components of the cranial base were transplanted (Fig. 1): the occipito-basisphenoid and the basi-presphenoid synchondrosis with a minimum amount of adjacent bone on both sides (*O* resp. *P*), and the same synchondroses with some more adjacent bone on both sides (*OB* resp. *BP*); cranial bases from the occipital foramen to the sphen-oethmoidal junction (*CB*) were also transplanted. The length, measured in the longitudinal direction of the cranial base, of the first two transplants was approximately 1.0 mm, that of the two other synchondrosis transplants 3.0 mm, and the length of the whole cranial bases varied from 9.0 to 10.5 mm.

Transplantation was always performed between littermates of the same sex. Pockets in the subcutaneous tissue were used as transplantation sites. The details of the technique have been described earlier (*Koski & Mäkinen*, 1963).

After periods ranging from two to 90 days (Table I) the hosts were killed by decapitation, the transplants were searched for, and, in cases of experimental periods of 15 or more days, measured *in situ* through the covering connective tissue capsule. All the transplants were then removed with a piece of the surrounding connective tissue and processed for histological examination as decalcified specimens stained with hematoxylin and eosin.

The number of transplanted pieces, recovered transplants, and the transplantation periods are given in Table I.

Table I.
Statistical data related to transplants

Type of transplant	Transplantation period, in days	Number of transplants	
		Original	Recovered
O & P	2	10	10
	5	17	17
	10	11	11
	15	46	45
	30	44	37
	60	38	34
OB & BP	2	10	10
	5	17	17
	10	11	11
	15	52	42
	30	49	45
	60	52	46
	90	18	16
CB	2	10	10
	5	12	12
	10	9	9
	15	11	11
	30	16	15
	60	28	22
		461	420

As can be seen, a number of transplants were not recovered. The great majority of the losses were due to very early death of the hosts, but in a few instances the transplants apparently had either been lost through the incision or had been resorbed, and could not be found at the termination of the experiment.

The dimensions of the histological specimens were checked from some slides and found to be considerably smaller than those of the same specimens recorded while still *in situ*. The differences were apparently mainly due to the effect of the covering capsule on the *in situ* measurements; the dimensions of histological control specimens (on slides) namely were in good agreement with the dimensions of the transplants taken before transplantation. Therefore, the *in situ* measurements were discarded and the small specimen series (*O, P, OB, BP*) were again measured from the histo-

logical slides, using a stereomicroscope furnished with an ocular scale, and read to the nearest 0.1 mm. The same could not be done with the large cranial base specimens, because the slides rarely contained good sections of the whole transplants. However, in many instances the distance between the midlines of the two synchondroses could be measured from the slides, and the same was done on a number of control specimen slides representing various age levels.

RESULTS

Macroscopic findings

The recovered transplants were, as a rule, found to be encapsulated and attached to the pannicular fascia. Their vascularization varied, and it appeared as if about 1/3 of the specimens would have been poorly vascularized, an impression which was not later verified during the histologic examination.

The dimensions of the measured specimens in the smallest transplant series (*O, P*) are given in Table II. The number of these specimens is somewhat smaller than that of recovered specimens; apparently some of the small specimens were lost during the process of histological preparation.

It can be noted that in the majority of instances no increase in length had occurred during transplantation; as a matter of fact, most of the transplants had decreased in length. In a number of cases some increase had taken place in the direction corresponding to the breadth of the cranial base.

As regards the transplants of the second main type (*OB, BP*), only three out of 147 inspected specimens were longer than originally, and none of them measured more than 3.5 mm in length. Two of these had been in the host for 15 days and one for 30 days.

Table II.

The rostro-caudad length of O & P transplants after varying periods of transplantation, indicated by numbers of specimens falling into dimensional categories.

Length in mm	Transplantation period in days						Total
	2	5	10	15	30	60	
≤ 1.0	10	14	5	27	29	16	101
1.1—1.5	—	2	—	7	5	7	21
1.6—2.0	—	—	1	2	3	2	8
2.1—2.5	—	—	—	1	—	1	2
Total	10	16	6	37	37	36	132

Table III.

The mean intersynchondroseal distances at various ages and after different periods of transplantation, in mm.

Specimen	Age of control animal / Period of transplantation						Mean increase
	2	5	10	15	30	60	
Control	—	2.9	3.7	4.3	4.9	6.1	3.2
Transplant	3.4	3.4	3.7	3.9	4.1	4.5	1.1
(N)	(10)	(9)	(8)	(7)	(6)	(6)	

As to the cranial base transplants, their size at the termination time varied from 6.5 to 12.5 mm; on an average, there was no increase in length. The number of specimens found to have increased in size was three out of 11 at 15 days, five out of 15 at 30 days, and 7 out of 22 at 60 days, but even in these instances the changes were quite small, considering the fact that the dimensions recorded through the capsule were overestimates of the true dimensions.

The results of the measurements between the midlines of the two synchondroses are given in Table III. The figures related to controls are based on measurements taken of histological median sagittal sections of whole skulls of three to five animals at each age. The short-term transplant dimensions are greater than the control dimensions; this, of course, is of no particular significance, since we are interested in the increase in length occurring within both samples separately. Since the mean and mode age of the donors was 5 days, the total life of the transplants is, on an average, 5 days longer than the age of the corresponding controls at each level, but the fact that the transplants go through an approximate five-day »shock» period in the beginning (see discussion) should justify the comparison in the way it is done here. As additional information it can be mentioned that the minimum difference between the intersynchondroseal distances at 5 days and at 60 days in the control sample was 2.6 mm, the maximum difference 3.9 mm, while the corresponding figures in the transplant sample were 0.5 mm and 1.8 mm, respectively.

It appears, then, that the intersynchondroseal length, i.e., then length of the basisphenoid, had increased in the transplants, on an average, but this increase was not nearly equal to that occurring *in situ* during the same period of time.

Microscopic findings

Synchondroses

At 2 days (*O, BP*): Cartilage appears vital; marrow (*BP*) drained of red cells. Around the transplants there are in most instances aggregates of inflammatory cells.

At 5 days (*O, BP*): Cartilage appears vital, the resting cell zone may be wider than originally. There is, in many instances, an invasion of the connective tissue elements of the host tissue into the transplants, which are becoming vascularized, and some osteoid formation is observed, probably originating from the invading osteogenic cells. In the host tissue around the transplants clusters of undifferentiated connective tissue cells are seen, and some transplants are being sealed off by osteoid tissue formed apparently by the osteogenic host cells (Fig. 2). The inflammatory reaction is subsiding. Osteoclasts are noticed in many instances both inside and, to a small degree, along the open edges of the transplants.

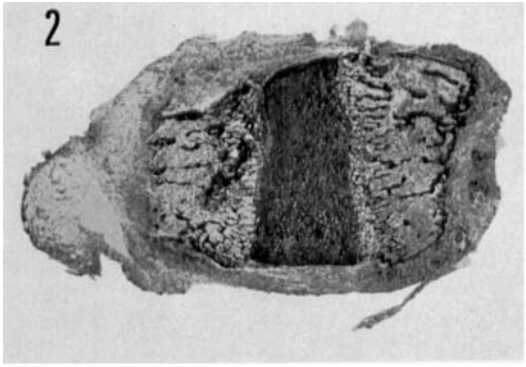
At 10 days (*O, BP*): Cartilage vital, the resting cell zone wider, row cell columns shorter than originally. Marrow is fibrous, osteoid formation observed in most instances. Osteoclasts present as above.

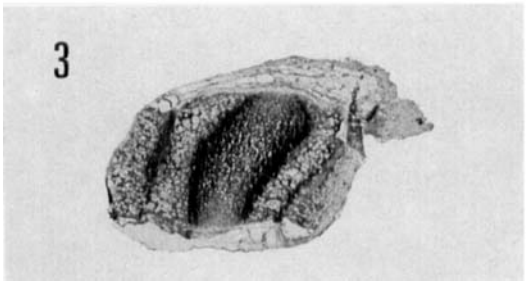
At 15 days (*O, OB, P, BP*): Cartilage vital, row cell columns still shorter, as are also the hypertrophic cell columns in many instances. Marrow fibrous or rather normal; in the latter cases the transplant edges, as a rule, are sealed off from the host tissue by bone. New bone formation in some instances inside the sealed transplants, with remodelling osteoclastic activity. In some cases aberrant growth with hypertrophic cartilage accumulating at the edges of the transplant. In a couple of specimens the original calcification front (?) is seen at a short distance from the present front, indicating some growth. One end of the transplant is sometimes longer than the other. Eight transplants in necrosis.

At 30 days (*O, OB, P, BP*): Cartilage vital, but loss of stain is noticed in most cases in the resting cell zone. Marrow is normal or fibrous; in the first cases the transplant is sealed off by bone. In some specimens primary spongiosa formation is seemingly taking place on the matrix remnants left from the receding cartilage. Some transplants exhibit aberrant growth with long columns of row and hypertrophic cells, necrotic bone spiculi, and fibrous marrow (Fig. 3). Six necrotic specimens.

At 60 days (*O, OB, P, BP*): Resemble those at 30 days. Lamellar bone is seen in many specimens, sometimes seemingly produced by the host cells (Fig. 4). Four transplants in necrosis.

At 90 days (*OB, BP*): The general picture as at 60 days; no necrotic specimens.





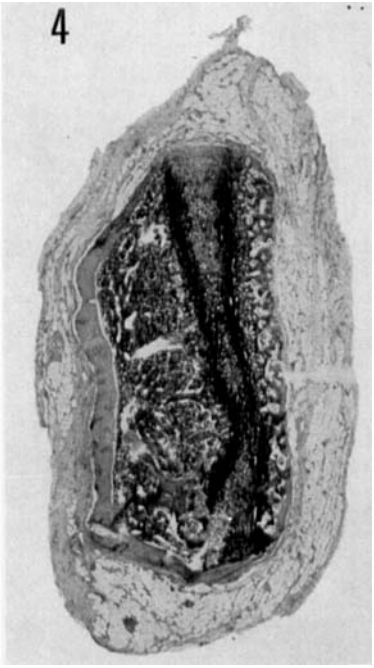


Fig. 2. Type BP transplant after 5 days. The resting cell zone probably wider than originally, marrow filled mainly by fibroblasts. At one end osteoid seal being formed by host cells. H & E, × 20.

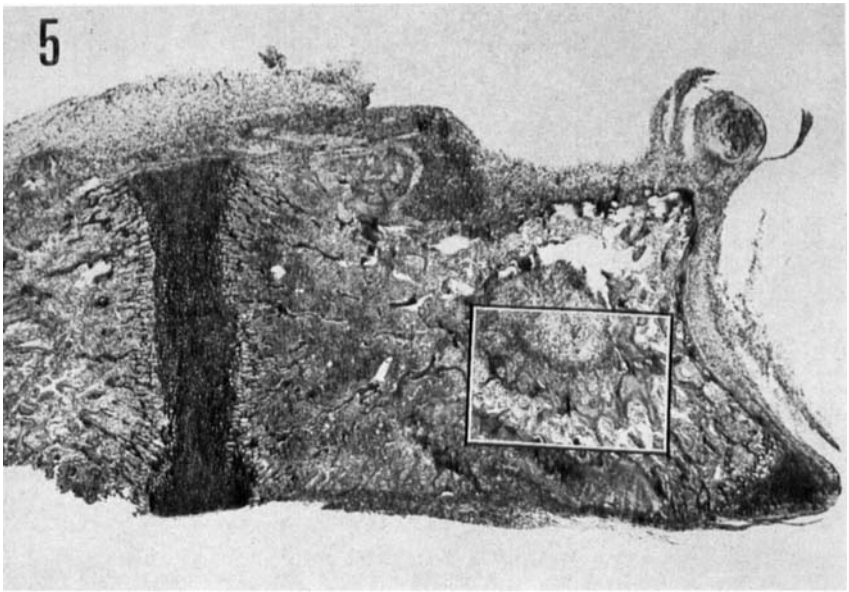
Fig. 3. Type P transplant after 30 days. Aberrant growth: the original row cell zones appear to have been »pushed» away by newly started proliferation, but primary spongiosa is lacking. H & E, × 20.

Fig. 4. Type OB transplant after 60 days. Narrow cartilage, normal »metaphysial» zone, and lamellar bone seal around the asymmetric transplant. Cartilage breadth (perpendicular to the long axis of cranial base) increased. H & E,× 20.

Cranial bases

At 2 days (Fig. 5): Cartilage cells shrunken, only a few red cells in the marrow, bone trabeculae partly vital, no osteogenic activity. Round cell aggregates around the transplants.

At 5 days (Fig. 6, 7): Cartilage cells still mostly shrunken, marrow fibrous or cellular, red cells appearing, original bone mainly necrotic, but very active new bone formation in some specimens. In one specimen cartilage vital, long hypertrophic columns. Round cell aggregates around some transplants. Four necrotic specimens.



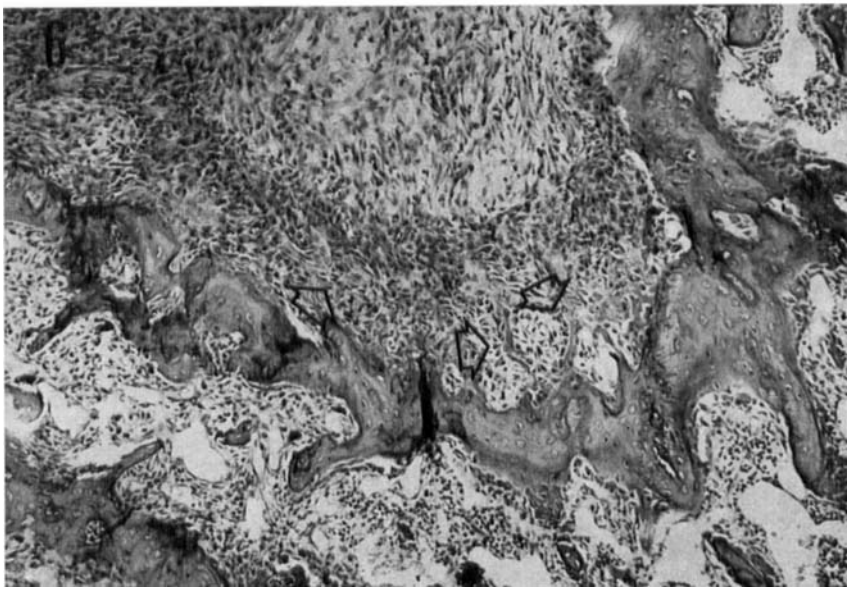


Fig. 5. The caudal portion of a cranial base transplant after five days. H & E, × 20.

Fig. 6. A part of the specimen in Fig. 5 at greater magnification. Note that some osteocytes in the original bone spiculi appear vital, and there is an aggregation of osteoprogenitor cells, of which osteoblasts have arisen and appear at newly formed bone spiculi (arrows). H & E, × 90.

At 10 days: Cartilage apparently vital in some specimens, not well stained in some. Endochondral growth seems to be taking place in some, marrow becoming normal in most specimens; bone vital, great osteogenic activity. In one specimen endochondral growth also at basioccipital cartilages. Round cells around the transplants seem to be disappearing. One necrotic specimen.

At 15 days: Cartilage vital, though poorly stained in resting cell zones; endochondral growth; marrow normal, bone vital, new bone formation turning lamellar in places; endochondral growth at some basioccipital cartilages. No round cell aggregations, except in one necrotic specimen.

At 30 days: Cartilage narrow, vital, though resting zone poorly stained, endochondral growth has taken place; marrow normal; bone vital, in many instances lamellar, osteoblastic activity from the marrow side. No host reaction, except in six necrotic cases.

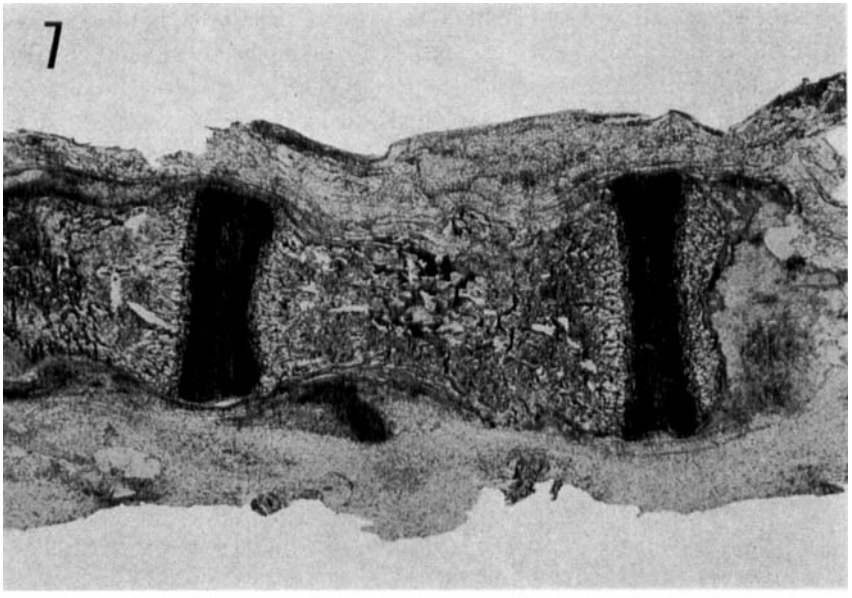
At 60 days (Fig. 8). As above; four necrotic specimens.

DISCUSSION

The present study represents a continuation of a series of experimental investigations into the nature of certain cranio-facial and other growth phenomena (*Koski & Mäkinen*, 1963; *Koski & Mason*, 1964; *Koski & Rönning*, 1965; *Rönning & al.*, 1967). In these investigations, the capacity of some so-called growth cartilages has been studied using the method of transplantation, with the idea that any independent potential that these cartilages may have for promoting bone growth should manifest itself also in non-functional environments capable of sustaining the vitality of transplants.

The fate of the transplants depends, among other things, on the effect of immunological and nutritional factors on them, as well as on the possible induced action of osteogenic cells of the host. These aspects have been discussed to some length in the previous papers, and will not be dealt with here. Since the experimental conditions in all the studies referred to above have been essentially similar, and since the number of transplants in every experimental series has been relatively great, the variability caused by the three factors mentioned should not seriously affect the comparison between the results of the different experiments.

The fate of the present transplants varied considerably. A number of them became necrotic or distorted in shape, yet the majority survived the transplantation well and were readily recognizable as synchondroseal cartilages or cranial bases at the time of the recovery.



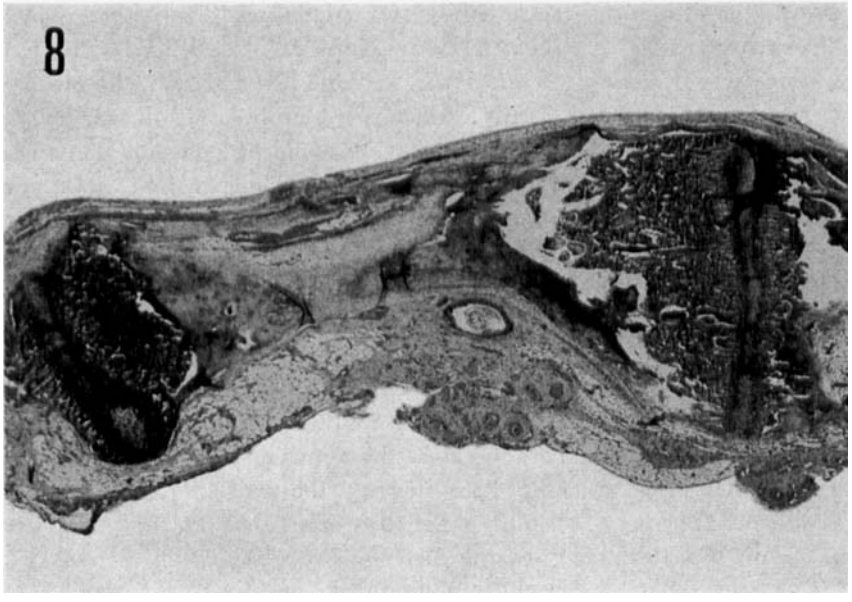


Fig. 7. A part of a cranial base transplant after two days. H & E, × 18.

Fig. 8. Middle portion of a cranial base transplant after 60 days. The intersynchondrosal distance is greater than in the specimen in Fig. 7 (same magnification). H & E, × 18.

The changes in the histological structure of the thriving transplants resembled closely those described by others working on similar problems (cf. *Felts*, 1961; *Chalmers & Ray*, 1962): There was first an early phase of »shock», affecting most elements of the transplant, followed by recovery which started before or around the fifth day of the transplant life, and resulted in new bone formation and regeneration of a hemopoietic marrow.

Regarding the independent bone-growth-promoting potential of the cranial base cartilages the information derived from the present experiments shows not a clear-cut trend. The mean increase in length of the transplants was nil in each series. However, some of the transplants had increased in length to some degree; how much of this increase could be attributed to the endochondral growth at the cartilages and how much of it was possibly due to the activity of the host osteogenic cells at the edges of the transplants, could not be ascertained. The most significant positive finding was that the intersynchondroseal distance increased in the cranial base transplants, even on an average.

Chalmers & Ray (1962) found that isologous mouse femur transplants attained about 80 % of the length of the femur *in situ* by 8–9 weeks; consequently, one would expect also transplants of the present type, which can perhaps be considered to be closer to isologous than homologous type, to remain somewhat shorter than the corresponding parts *in situ*. As far as the cranial bases are concerned, nutritional difficulties could also be of some importance in these relatively large transplants; however, since the transplants, in the majority of cases, became well vascularized and some growth was observed within them the effect of the nutritional difficulties is not clear. The effect of the host tissue capsule especially on these large transplants is also unknown.

The cranial base of the rat nearly doubles its length at birth by the 30th day, and is more than twice the birth length by the 60th day (*Baer*, 1954). The transplanted cranial bases exhibited only a minimal amount of total growth, and even the observed maximum increase in the intersynchondroseal distance, i.e., in the basisphenoid length, was less than 50 % of the corresponding increase taking place *in situ*. The sum of the maximal increases in length of the two synchondroseal transplants during 60 days of transplantation was 2.0 mm; thus it would not suffice to explain the growth occurring in the cranial base *in situ*, which amounts to about 9 mm from the fifth to the 60th day of life (*Baer*, 1954).

In trying to evaluate the dimensional observations, it seems justified to conclude that the rat cranial base synchondroses, when transplanted subcutaneously, do not manifest the growth potential that could be expected

on the basis of *in situ* observations. When the growth of these transplants is compared to that of the cartilaginous epiphyseal ends of long bones under similar experimental conditions, the difference is quite obvious, the latter exhibiting a four-to-fivefold increase in length (*Koski & Rönning*, 1966).

In this connection attention might be drawn to the asymmetry observed in many synchondroseal transplants, i.e., there was more bone on one side of the cartilage than on the other. This brings up a question: does there exist a degree of polarity in these cartilages regarding their potential for endochondral bone formation? It can be mentioned that a similar asymmetry as that found here was observed in some intracerebrally transplanted cranial base cartilages (*Koski & Rönning*, unpublished data). Further investigations into the proliferation rate of the cartilages *in situ* should bring an answer to this question.

Histologically, endochondral bone formation occurred in many instances within the transplants in each category, even if the overall length of the transplant did not change. The cartilages became narrower with age, and the adjacent areas, which originally had consisted of marrow and only a few bone spiculi, became more densely filled with newly formed spiculi. The histological changes thus resembled those occurring *in situ* with advancing age, and indicated some degree of bone-growth-promoting potential in the synchondroseal cartilages.

In their organ culture experiments *Petrovic & Charlier* (1967) found that the synchondroseal cartilages grew to about 80 % of the length of the controls, but new bone formation was almost non-existent, the increase in length being mainly due to the growth of the cartilage. On the other hand, mandibular condyle cartilages did not exhibit any growth under similar experimental conditions (*Charlier & Petrovic*, 1967). These authors' conclusion that there is independent growth potential in the synchondroseal cartilage but not in the condylar cartilage, seems to be in agreement with the results obtained in the present study and in the previous investigations on the condylar cartilage transplants (*Koski & al.*, 1963, 1964, 1965).

It may be of importance, however, to distinguish between the independent growth potential of the cartilage tissue itself and its potential to promote bone growth. It may, furthermore, be important to make a distinction between a mere calcification of cartilage tissue, growing or not growing in size, and its ability to participate in typical endochondral bone formation as seen in the long bones. Finally, the environment of the cartilage growing in ectopic sites, both in *in vivo* and *in vitro* experiments, may have an effect on the manifestation of the growth potential; e.g., it seems as if the growth of synchondroseal cartilage transplants in the brain tissue would

be of greater magnitude than its growth in subcutaneous sites (*Koski & Rönning*, unpublished data). Until further information about this aspect is available, it might be advisable not to compare results of experiments of different design.

When compared to the condylar cartilage and the epiphyseal cartilaginous ends of long bones, the synchondroseal cartilage seems to occupy a position between these two, regarding the independent bone-growth-promoting potential. The synchondroses and the cranial bases maintain their microscopic structure much in the way the epiphyseal cartilages do, while the condylar cartilages become disorganized, in the transplant sites. On the other hand, the synchondroses do not show the same amount of dimensional growth as the epiphyseal cartilages. This may be expected, since there is a difference between the synchondroses and the epiphyseal growth cartilage in that the length of the cell columns is much less in the former than in the latter, (*Rönning & al.*, 1967), and, at least in the long bones, there seems to be a correlation between the length of the cell columns and the growth rate in length of the diaphysis (*Hansson*, 1967).

The tentative conclusion that appears justified on the basis of the results of the present study and the information available from previous sources is that the cranial base synchondrosis possess a bone-growth-promoting potential of its own, which, however, is not equal to that of epiphyseal cartilages of long bones. It may well be that in order to fully manifest its inherent potential the cranial base cartilage needs the stimuli provided by its natural environment.

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SUMMARY

A study of subcutaneously transplanted cranial base synchondroses and cranial bases in rats has resulted in the following findings and conclusions:

(1) The majority of transplants survived the transplantation and exhibited rather normal histology up to 90 days.

(2) The dimensional growth of the transplants was, on an average, nil, but in some cases increase in size occurred.

(3) The bone-growth-promoting potential of the cranial base synchondroses appears not to be equal to that of epiphyseal end cartilages of long bones.

RÉSUMÉ

POTENTIEL DE CROISSANCE DES SYNCHONDROSES DE LA BASE DU CRÂNE DU RAT APRÈS TRANSPLANTATION SOUS-CUTANÉE

Les résultats et les conclusions suivantes ressortent d'une étude sur la transplantation sous-cutanée de synchondroses de la base du crâne et de bases du crâne entières chez le rat:

(1) La majorité des transplants survivaient à la transplantation et présentaient un aspect histologique à peu près normal pendant une période allant jusqu'à 90 jours.

(2) La croissance des dimensions des transplants était en général nulle, mais, dans certains cas, on constatait une augmentation de leur grandeur.

(3) Le potentiel favorisant la croissance osseuse des synchondroses de la base du crâne semble donc ne pas atteindre le niveau de celui des cartilages de conjugaisons des épiphyses dans les os longs.

ZUSAMMENFASSUNG

Das Wachstumspotential der in das Subkutis transplantierten Synchondrosen der Schädelbasis bei der Ratte.

Aus einer Studie ueber die in das Subkutis transplantierten Synchondrosen der Schädelbasis und ueber die Schädelbasen selbst ergaben folgende Beobachtungen und Schlussfolgerungen:

1. Die Mehrzahl der Transplantaten ueberlebten die Transplantation und zeigte eine ziemlich normale Hisrtologie bis zum 90. Tag auf.

2. Durchschnittlich betrachtet kann von dem Wachstum der Transplantatten keine Rede sein aber jedoch konnte in einigen Fällen Zunahme der Grösse festgestellt werden.

3. Die Synchondrosen der Schädelbasis scheinen nicht die gleiche das Knochenwachstum befördernde Fähigkeit zu besitzen wie die epiphysealen Knorpeln der Röhrenknochen.

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