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AN EXAMINATION OF GRAFT ALTERATION AND RECIPIENT RESPONSE TO PROCESSED MARE CORTICAL BONE XENOGRAFTING

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The examination was conducted in order to investigate the effectiveness of horse bone xenografting and to compare the differences in various processed bone graft. The grafts of mare cortical bones processed by boiling, freezing, and deproteinization were implanted into the humeral bone marrow of adult mongrel dogs and fresh grafts were used as controls. The results were summarized as follows.

1) By roentgenographic examination, the bone graft density was increased quickly in order of deproteinized, frozen, boiled, and fresh graft.

2) By gross appearance, the ossal fusion between the graft and the recipient was completed in all deproteinized and frozen grafts and in a few boiled and fresh graft.

3) The bone replacement in graft was not found up to 16 weeks after implantation in all the processed and fresh grafts.

4) The major bone mineral contents at the end of 16 weeks after implantation were nearly in agreement with the absorption rate in each graft.

5) There were no changes on blood cells and serum electrophoretic fractions of recipients implanted with non-processed fresh graft.

INTRODUCTION

The use of bone grafting depends on (1) the immediate supporting effect, (2) the character and amount of reparative tissue which the graft stimulates the recipient to form (inductive ability), and (3) the reproduction of new tissue by itself (osteogenic potency)[2]. The bone autografting certainly serves the above purposes. It is often difficult to obtain the bone materials for autograft as well as homograft. Recently, the bone xenografting is applying clinically. Deproteinized calf bone graft in various processed bone grafts is very common on its use for man. Horse bone has a great amount of bone tissue and is easily obtained as cattle bone, but horse bone grafting has been little investigated.

The purpose of the present study is to evaluate horse bone grafting for clinical use.

MATERIALS AND METHODS

Dogs

Twenty healthy adult mongrel dogs were divided into four groups of five. The following various processed bone grafts were implanted into the humeral bone marrow of dogs of each group.

Bone graft

All grafts, which were 2×2×50 mm in size, were made from mare cortical bone. The boiled bone grafts were made by boiling for 20 minutes, the deproteinized bone grafts made by Kobe Bone method[18] and the frozen bone grafts stored for 1 month at −20°C. The fresh bone grafts (G 1), the boiled bone grafts (G 2), the deproteinized bone grafts (G 3) and the frozen bone grafts (G 4) were implanted completely into the right humeral bone marrow through its *Tuberclum majus*.

Observation

The fate of the graft was examined by roentgenogram at 1 hour and 4, 8, 12, and 16 weeks after implantation. After 16 weeks, the graft were taken out from its recipient to gross examination. For measurement of both the dry bone and the ash weight of the graft, the callus and bone marrow tissue around the graft were taken away carefully to return to its original form. The contents of calcium (Ca), magnecium (Mg), and inorganic phosphorus (P) of the each graft were determined by the chelate titration method.

Packed cell volume, erythrocyte count, leukocyte count, differential leukocyte count, and serum electrophoretic fractions were examined. Electrophoretic fractions in serum protein were examined by cellulose acetate electrophoresis. The above determination were performed before implantation (T 0), on 1st (T 1), 7th (T 2), 13th (T 3), 19th (T 4), 25th (T 5), and 31st days (T 6) after implantation.

The results were analyzed statistically using one way analysis.

RESULTS

Roentgenographic findings (figs. 1～4)

In all 5 cases in group 1, the shadow around graft and the increased bone graft density were little found at 16 weeks after implantation.

In a few cases in group 2, the shadow around proximal end of graft and the increased bone graft density at its proximal end were begun to appear from 8 or 12 weeks after implantation.

In all cases in group 3, the increased bone graft density appeared from 4 or 6 weeks after implantation. The increase of shadow was inversely proportional to the increase of bone graft density.

The findings in group 4 were the same as in group 3.

Gross examination (figs. 5~9 & tab. 1)

In all cases of group 1 and 2, the new bone formation (spongiosa) around graft was slightly found and the surface of graft was little absorbed.

In all cases of group 3, the spongiosa was formed completely around the graft and the ossal fussion between graft and recipient was found. The bone marrow tissue of recipient developed into the spongiosa around graft and the increased absorption of graft was found to be proportional to increase of the spongiosa around graft. Most grafts in group 3 were so absorbed that they were altered into spongy.

The findings in group 4 were the same as in group 3.

TABLE 1 *Gross findings of grafts*

GROUP	G 1					G 2				
No. OF DOG	B 1	B 2	B 3	B 4	B 5	B 6	B 7	B 8	B 9	B10
Deposited spongiosa on grafts	+	+	+	+	+	+	+	++	+	++
Absorption of grafts	+	+	−	−	−	+	+	++	+	+
GROUP	G 3					G 4				
No. OF DOG	B11	B12	B13	B14	B15	B16	B17	B18	B19	B20
Deposited spongiosa on grafts	+++	+++	+++	++	++	+++	+++	++	++	+++
Absorption of grafts	+++	+++	++	+++	+++	+++	++	++	++	+++

Weight and mineral contents (tab. 2)

The weight of dry bone graft among each graft was found marked distinction. Particularly, the weight of dry bone graft of group 3 was one half that of group 1. That distinction was encountered much the same with the ash weight and Ca, Mg, and P contents of graft among each group. Each group showed the decrease of both dry bone and ash bone weight of graft at 16 weeks after implantation in the following order : group 3, group 4, group 2, and group 1. The percentage of Ca, Mg, and P contents per ash weight of graft among each group after implantation was found a certain variation. Therefore, it may be suggested that there was slightly difference in these absorption rates.

TABLE 2 *Weight of dry bone, ash and mineral elements of grafts before and after implantaiton*

	GROUP	DRY BONE	ASH	MINERAL ELEMENTS		
				Ca	P	Mg
		mg	mg	mg	mg	mg
After implantrtion	G1	420.13	277.91 (66.2)*1	84.05 (30.3)*2	37.22 (13.4)*2	0.80 (0.29)*2
	G2	371.10	274.05 (66.6)	72.28 (29.3)	75.52 (30.6)	0.74 (0.30)
	G3	215.95	131.89 (61.1)	48.17 (36.5)	47.74 (34.7)	0.65 (0.51)
	G4	258.39	169.83 (65.7)	69.76 (41.1)	65.01 (38.3)	0.78 (0.46)
Before implantation	G1	429.29	284.50 (66.3)	109·79 (38.6)	80.70 (28.4)	1.81 (0.64)
	G2	431.55	292.22 (69.3)	142.69 (47.7)	86.98 (29.1)	2.70 (0.90)
	G3	430.02	282.42 (65.7)	132.83 (47.0)	49.34 (17.5)	1.86 (0.59)
	G4	429.55	290.36 (67.6)	109.48 (37.7)	87.11 (30.0)	2.45 (0.84)

Remarks: *1 Ash weight/dry bone weight %
*2 Mineral elements weight/Ash weight %

Hematological findings (tabs. 3 & 4)

The each value of blood findings was not significantly different among 4 groups.

The results on time-course (T 0 …… T 7) of each value were as follows. The packed cell volume was decreased at T 1 and T 5 compared with T 0. There was a highly significant difference ($P<0.01$).

The decreased leukocyte count was found a little after implantation, but there was no significant difference. On the time-course after implantation variation of segment III of neutrophil in differential leukocyte count was found. The increased segment III count was found since T 1. The difference among them was significant ($P<0.05$) at T 1 and highly significant ($P<0.01$) since T 2.

The total serum protein was decreased progressively after implantation in all four groups. But these values were within the normal range and there was no significant difference.

The electrophoretic fractions in serum protein and A/G ratio were not significantly different.

In the β-globulin of the electrophorogram (fig. 10), monophasic pattern (A type), diphasic pattern (B type) in globulin and other pattern (C type) except A and B type were found as described in table 5. There was no regulation in variation of their pattern in β-globulin.

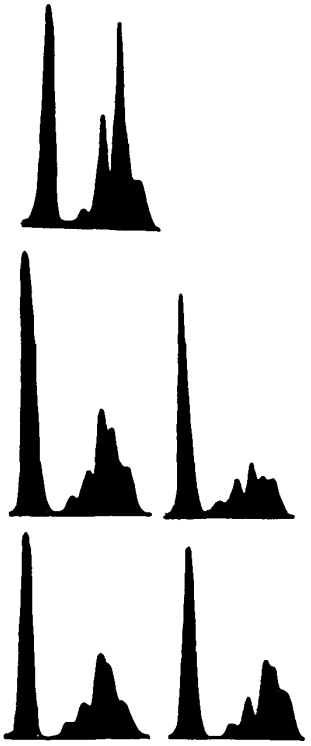
TABLE 3 *Hematologic findings with time course before and after implantation in dogs*

GROUP	TIME	PACKED CELL VOLUME	ERYTHROCYTE	LEUKOCYTE	DIFFERENTIAL LEUKOCYTE COUNT						
					Basophil	Eosinophil	Lymphocyte	Monocyte	Neutrophils		
									Band	Segment	
										II	III
		%	$\times 10^6$/mm^3	$\times 10^3$/mm^3	%	%	%	%	%	%	%
	T 0	46.4	7.06	7.51	0.	7.9	12.4	10.2	2.1	2.4	65.0
	T 1	40.2	6.01	10.38	0.	4.5	10.3	12.0	1.4	1.6	70.2
	T 2	41.9	7.28	10.00	0.	9.2	12.3	14.4	1.5	1.7	60.9
G 1	T 3	40.7	6.29	7.78	0.	8.3	14.8	11.6	2.0	2.1	61.2
	T 4	36.9	5.76	8.75	0.	10.6	16.8	7.9	1.7	2.0	61.0
	T 5	36.8	5.60	9.51	0.	10.7	18.4	9.3	2.0	2.1	57.5
	T 6	40.1	6.34	7.44	0.	11.3	14.2	8.5	2.2	2.3	61.5
	T 0	44.7	6.79	7.69	0.	13.1	8.7	13.1	2.2	2.6	60.3
	T 1	42.6	6.30	10.18	0.	7.9	7.0	12.3	1.7	1.8	69.3
	T 2	39.5	6.11	10.51	0.	9.9	18.1	13.8	2.3	2.6	53.3
G 2	T 3	42.6	6.44	6.85	0.	12.7	15.3	12.9	2.5	2.8	53.8
	T 4	44.3	6.76	8.33	0.	6.0	13.6	14.8	2.1	2.3	61.2
	T 5	41.0	6.34	7.03	0.	13.0	13.5	14.1	2.6	3.1	53.7
	T 6	42.4	6.97	7.14	0.	12.4	16.1	9.1	2.1	2.4	57.9
	T 0	40.6	6.51	9.50	0.	9.3	11.8	18.9	1.6	1.7	56.7
	T 1	33.9	7.43	11.10	0.	7.3	13.9	9.4	1.2	1.3	66.9
	T 2	38.1	6.04	7.99	0.	14.9	14.5	11.9	2.0	1.9	54.8
G 3	T 3	38.1	6.12	9.34	0.	8.3	13.2	12.1	1.7	1.9	62.8
	T 4	38.9	6.28	7.85	0.	12.5	12.6	10.8	2.2	2.3	59.6
	T 5	36.2	5.79	6.70	0.	14.8	13.1	10.2	2.2	2.3	57.4
	T 6	39.0	6.15	8.59	0.	13.3	13.1	11.8	2.0	2.0	57.8
	T 0	40.0	5.73	7.73	0.	2.0	12.2	12.0	2.3	2.3	69.2
	T 1	38.9	5.48	7.68	0.	8.4	12.3	9.8	1.8	2.3	65.4
	T 2	40.2	5.92	8.57	0.	6.1	16.3	10.0	1.6	2.0	64.0
G 4	T 3	39.4	5.52	8.24	0.	5.3	24.2	8.8	1.8	1.6	58.3
	T 4	41.7	6.08	8.55	0.	9.3	26.3	6.8	1.7	2.1	53.8
	T 5	41.2	6.05	8.81	0.	10.0	24.0	8.2	1.8	1.8	54.2
	T 6	41.0	6.21	8.49	0.	7.5	17.2	10.7	1.9	2.0	60.7

TABLE 4 *Total serum protein, electrophoretic fractions and albumin/globulin ratio with time course before and after implantation in dogs*

GROUP	TIME	TOTAL SERUM PROTEIN	ELECTROPHORETIC FRACTIONS					A/G ratio
			Albumin	Globulin				
				α 1	α 2	β	γ	
		gm/dl	%	%	%	%	%	
	T 0	6.92	39.3	9.3	16.9	22.1	12.4	0.647
	T 1	7.12	37.8	9.5	17.5	22.7	12.5	0.608
	T 2	6.56	38.8	10.8	16.2	21.7	12.5	0.634
G 1	T 3	6.48	39.7	7.4	15.2	23.3	14.4	0.658
	T 4	6.38	36.6	6.4	16.5	30.0	10.5	0.577
	T 5	5.98	39.8	7.0	14.5	27.0	11.7	0.661
	T 6	6.20	39.6	8.8	15.3	23.8	12.5	0.656
	T 0	6.74	42.4	8.6	14.8	23.0	11.2	0.736
	T 1	6.66	38.0	8.5	14.1	24.8	14.6	0.613
	T 2	6.20	39.9	7.7	14.2	24.5	13.7	0.664
G 2	T 3	6.32	43.4	7.2	12.3	22.9	14.2	0.767
	T 4	5.84	42.7	7.1	15.8	27.8	6.6	0.744
	T 5	6.00	43.4	8.8	13.6	22.3	11.8	0.767
	T 6	5.92	42.6	9.0	14.2	21.2	13.0	0.742
	T 0	6.30	42.4	8.0	12.7	26.5	10.4	0.736
	T 1	6.18	41.5	7.6	14.2	26.2	10.5	0.709
	T 2	6.36	42.8	6.5	12.4	30.8	7.5	0.749
G 3	T 3	6.64	37.4	9.2	13.7	27.1	12·6	0.597
	T 4	6.72	41.6	6.4	11.6	29.4	11.0	0.712
	T 5	5.92	39.6	7.7	12.7	26.5	13.5	0.656
	T 6	6.30	39.5	8.9	13.9	25.7	12.0	0.653
	T 0	6.40	37.1	8.4	14.4	25.7	14.4	0.590
	T 1	6.10	38.0	7.2	15.7	25.1	14.0	0.613
	T 2	6.68	38.8	8.9	15.3	24.1	12.9	0.634
G 4	T 3	6.64	39.5	8.8	15.8	23.6	12.3	0.653
	T 4	6.72	40.3	8.0	15.5	23.4	12.8	0.675
	T 5	5.92	39.6	7.8	16.0	25.4	11.2	0.656
	T 6	6.30	39.3	7.7	15.0	25.6	12.4	0.647

FIGURE 10 *Electrophorogram of serum protein*



A type : monophasic pattern in β-globulin

B type : diphasic pattern in β-globulin

C type : other pattern than A and B type in β-globulin

TABLE 5 *β-globulin patterns in electrophorogram with time course after implantation*

GROUP	NO. OF DOD	TIME						
		T 0	T 1	T 2	T 3	T 4	T 5	T 6
G 1	B 1	+	*	*	+	*	−	−
	B 2	+	+	+	+	*	*	*
	B 3	+	+	+	+	+	+	*
	B 4	+	*	*	*	+	*	−
	B 5	*	+	+	+	*	*	−
G 2	B 6	−	−	+	−	−	*	*
	B 7	+	+	*	*	+	−	+
	B 8	−	−	−	−	*	+	+
	B 9	+	+	+	+	+	+	+
	B10	+	−	+	*	+	*	*
G 3	B11	*	+	+	+	+	+	+
	B12	+	*	+	+	+	+	+
	B13	−	*	−	−	−	−	−
	B14	+	+	+	*	*	*	*
	B15	*	*	−	*	*	−	*
G 4	B16	*	+	+	+	+	+	+
	B17	*	+	+	+	+	+	+
	B18	*	+	+	+	+	+	+
	B19	*	*	*	*	+	+	+
	B20	*	*	+	+	+	+	+

Remarks: − : A type + : B type * : C type

DISCUSSION

The antigenicity of homogenous bone was so low that the osteogenesis of bone homograft as well as bone autograft itself was often produced[2,6,8]. INCLAN (1942) reported success in the homograft preserved in a refrigerator at+2°C to +5°C, BUSH & GARBER (1948) recommended the Bone Bank to use the homogenous frozen bone. On the other hand SENN (1889) reported success in the decalcified ox bone xenografting. KRANACHER (1897) observed roentgenographically the new bone proliferation surrounded the calf bone xenograft boiled in soda solution or preserved in carboalcohol solution. JUDET & ARVISET (1949) reported a successful result in the frozen calf xenograft. SINGH et al. (1973) compared the KIRSCHNER intramedullary pins with macerated buffalo bone graft in beneficial effect of immobilization for repair of canine fracture, and obtained the result that this bone xenograft was more effective.

The organic components are not favorable for a successful bone xenograft compared to the mineral substances in bone tissue. The homogenous bone is low antigenic, but the organic components containing bone antigen effectively stimulant the osteogenesis of recipient[18]. SAMMORI (1951) reported that the decalcified bone homograft was more useful than the deproteinized, because of the rapid ossal replacement in graft.

As the antigenicity of bone xenograft plays a role of foreign body, the bone xenograft is usually unsuccessful. Thus the xenogenous bone has been processed to produce the lower antigenicity. At present, the deproteinized bone graft is most significantly effective among various processed bone graft; boiled, frozen, decalcified[27], and macerated by various solution[26]. Recently, the deproteinizable calf bone has generally used in xenograft of man so that the absorption and replacement of graft and new bone formation, ossal fusion between graft and recipient, occurred rapidly and effectively[18]. During the method of deproteinization, MAATZ & BAUERMEISTER (1957) employed hydroperoxide in order to remove the organic components from bone and LOSSEE & HUREY (1956) employed ethylendiamin for the same purpose. Moreover, Kiel Bone[16] and Boplant were made by removing the lipid of bone by ethel and Kobe Bone was to improve the above procedure. The nitrogen content rate of Boplant, Kobe Bone, and Kiel Bone is 5.56%, 2.12%, and 1.23% respectively[18]. The bone became fragile after lowered nitrogen content in bone[18]. It was described that a small quantity of nitrogen content in Kiel Bone was not enough to stimulate the osteogenic potency in recipient. On the other hand, the more nitrogen content and density of bone tissue, the more the vascularization which triggers absorption of bone graft and replacement of bone is delayed[18]. The vascularize-

tion in cancellouse bone graft of Kobe Bone was similar to that in bone autograft[19].

By roentgenographic findings in this examinations, the grafts was absorbed quickly in the order of deproteinized, frozen, and boiled grafts. The absorption of graft was depended on antigenicity. Fresh grafts were little absorbed. This result nearly agreed with the results which many investigators[7,11,19,26] obtained by the examination of xenografting employed the various processed bone grafts. Comparing the fate of bone graft which was placed in the bone marrow with the fate of other site ; subcutaneously, in muscle, and on osseous tissue, there were certain difference in the degree of absorption velocity or rate of graft, the ossification around the graft, and the ossal fusion between graft and recipient. By roentgenographic findings deproteinized bone graft placed on the site of artificial defect on osseous tissue, the absorption and replacement of bone graft were found at about 4 weeks after transplantation and the ossal fusion between graft and recipient was completed at about 10 weeks after transplantation. Ossal fusion between fresh bone graft and recipient was not found at 6 weeks after transplantation[7,11,18,19,21,26]. Grossly, as being possible to predict by roentgenograhpical findings, the deproteinized bone graft was the most absorbed one and the bone marrow tissue intruded in to the absorbed graft. But the bone replacement in graft was not yet appeared grossly at 16 weeks after implantation and the graft remained unchanged. But the ossal fusion between the graft and recipient, that in the callus formation around the graft was found.

The absorption and the callus formation around the graft were arranged in order of increasing rate in gross appearance of graft : deproteinized, frozen, boiled, and fresh graft. WEINMAN suggested that the inorganic salts had sufficiently the stimulating effect for the osteogenesis in recipient on the ossal fusion between graft and recipient with the advance of ossification around the graft and that was supported by NOBUHARA (1965). The character and amount of reparative tissue which the inorganic salts stimulate the recipient to form, that is major purpose of bone grafting, was presented sufficiently. It may be regarded as the important factor which the ossification around the graft and ossal fusion were promoted by the latent osteogenic capability in the bone marrow[4,5,24]. As already described, there was marked difference in the absorption rate between the methods for processing graft. This difference can be attributed to the various antigenicity caused by various processing methods. It is considered that the bone matrix of deproteinized graft is degenerated by deproteinizing process, resulting in the vascularization quickly.

KAWAMURA et al. (1962) investigated the absorption manner in graft by employing inorganic phosphorus (^{32}P) and concluded that ^{32}P transferred selec-

tively from graft to recipient through the blood flow and at the same time it transferred diffusely from graft to recipient and from recipient to graft at the local point. This results obtained were nearly in agreement with the predicted phenomenon that the callus formation around graft may increase along with the absorption of graft. The mineral content values in graft at the end of the 16 weeks after implantation were not only residual mineral content after absorption but also they transferred diffusely from the recipient. Therefore, the difference in the rate of the absorption and transference from the recipient concerning the correlation among Ca, Mg, and P could not be concluded.

It had been conceived that the bone grafting induced mild fever on the living body. Until now the object of study on examined bone grafting had been almost the immunological studies or the cure of grafted local point. The hematological and clinical findings in this study proved the above empirical fact. On the leukocyte count the momentary variation by operating detriment was present but that variation was ranged within normal value. There was no significant variation in eosinophil count which responded sensitively to detriment on body. However, the slightly increased segment III count in neutrophil was found. It was, therefore, expected that the living body might perform protective control. Increase in leukocyte count was clinically major index for the reject phenomenon on organic transplantation. Therefore, it is considered that the bone grafting has little influence on the hematological findings. SIBANO (1958) found the increased γ-globulin in electrophoretic fractions and the increased antibodies in the sera by the hemagglutination assay with the extracted antibodies of bone. TANNO et al. (1967) proved antibodies in the sera by hemagglutination assay and MILLONIG et al. (1962) by immunoelectrophoresis. The increased protein fractions which contained antibodies in cellulose acetate electrophoresis were at least not found in this study. Therefore, it was considered that there was little effects on the variation of electrophoretic fraction of the fresh horse bone or its processed bone grafting. The increased β-globulin was found in dog serum protein infected the hookworm disease or horse immunized by tetanic toxin and in its electrophorogram the diphasic pattern in β-globulin appeared (fig. 10)[25]. In this study, the variation pattern in β-globulin with time-course had no regularity.

It is considered that the antigenicity of horse bone is very low for dog.

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EXPLANTATION OF PLATES

PLATE I Figs. 1~4 are X-ray findings after implantation.

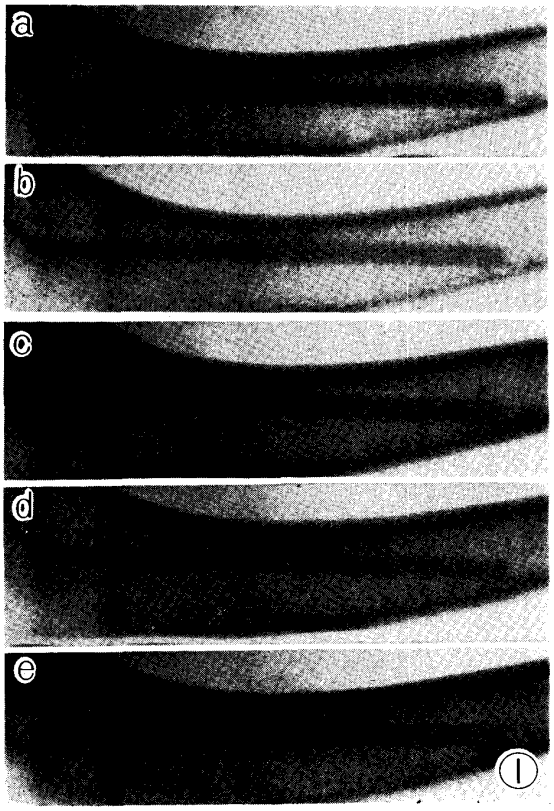
Fig. 1 The appearance of shadow around graft and the increased bone graft density was little found at 16 weeks after implantation. Case No. B 5 in Group 1

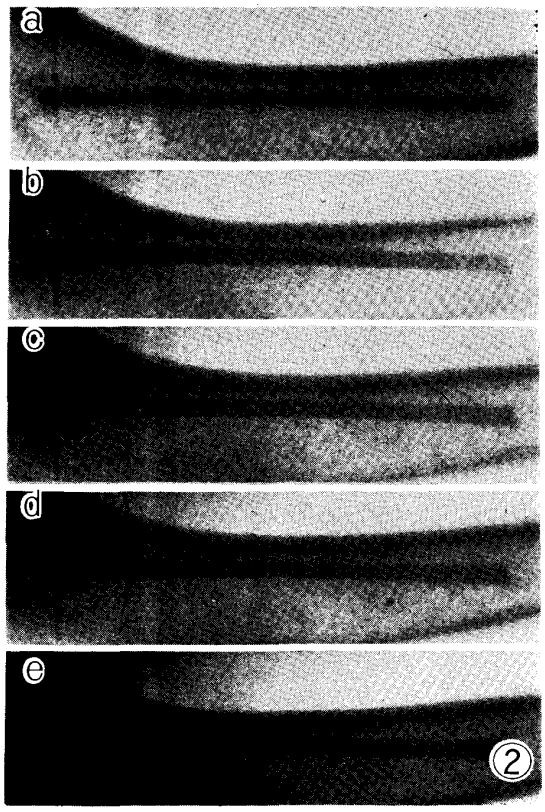
Fig. 2 The appearance of shadow around approximalis of graft and the increased bone graft density at its approximalis was begun to appear from 8 or 12 weeks after implantation. Case No. B 7 in Group 2

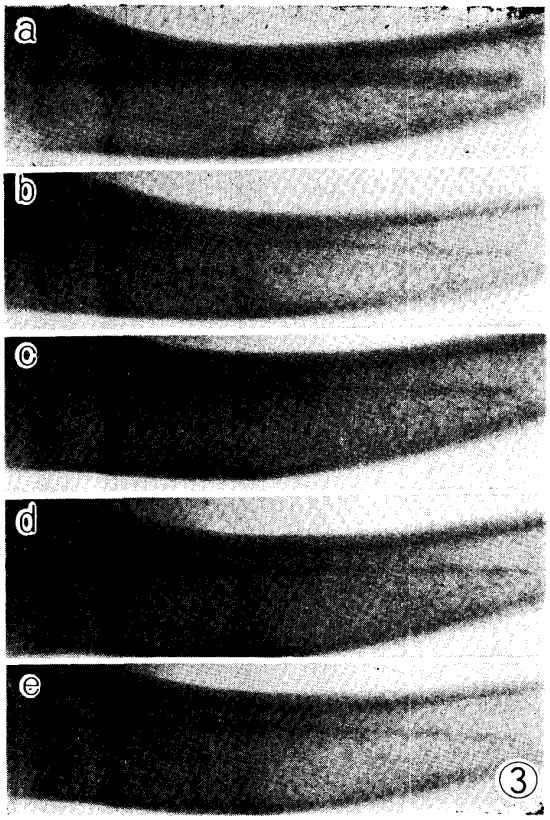
Fig. 3 The increased bone graft density was found from 4 or 6 weeks after implantation and the edge of graft was slightly found at 16 weeks after implantation. The shadow around graft increased inversely proportional to the increased bone graft density. Case No. B 13 in Group 3

Fig. 4 The findings of G 4 was found as same as fig 3. Case No. B 20 in Group 4

a : 1 hour after implatation
b : 4 weeks after implantation
c : 8 weeks after implantation
d : 12 weeks after implantation
e : 16 weeks after implantation


a
b
c
d
e
1


a
b
c
d
e
2


a
b
c
d
e
3

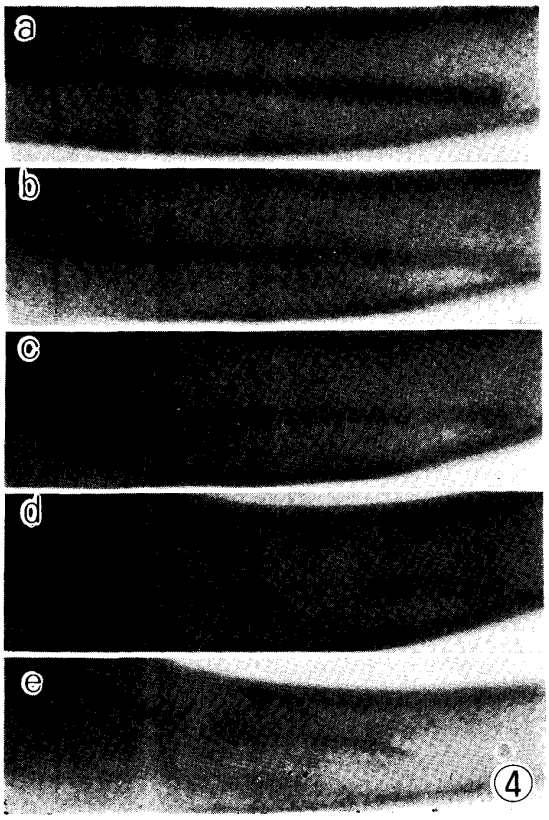

a
b
c
d
e
4

PLATE II Figs. 5~9 are gross findings of grafts at 16 weeks after implantation.

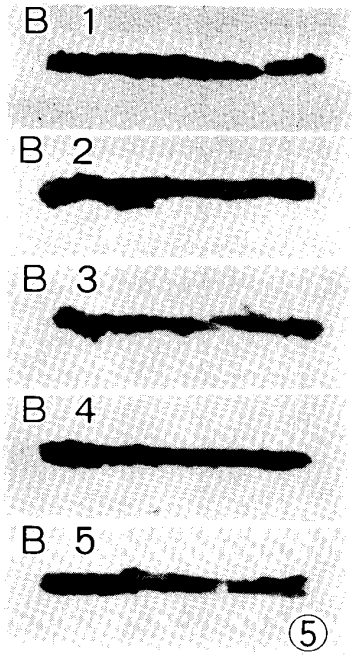
Fig. 5 Spongiosa around the grafts was slightly found and surface of the grafts was little absorbed. Case Group 1

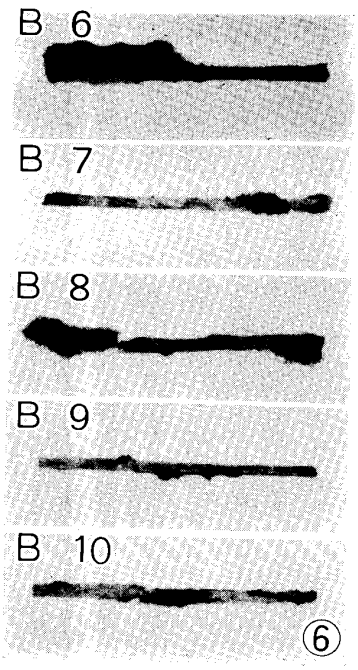
Fig. 6 They were found as same as group 1. Case Group 2

Fig. 7 Spongiosa around the grafts was found wholly around the grafts and the grafts were absorbed as same as spongy. Case Group 3

Fig. 8 They were found as same as group 3. Case Group 4

Fig. 9 Enlarged photograph of Case No. B 13 in group 3


B 1
B 2
B 3
B 4
B 5
⑤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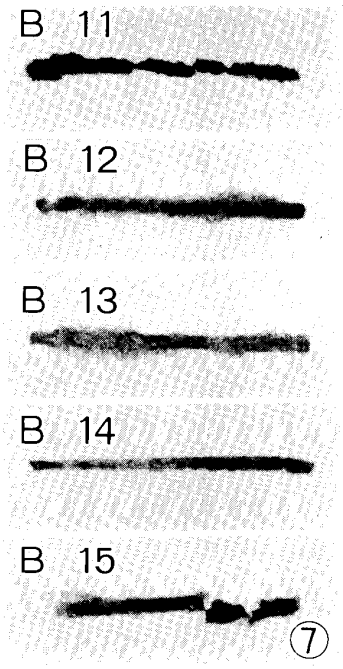
B 7
B 8
B 9
B 10
⑥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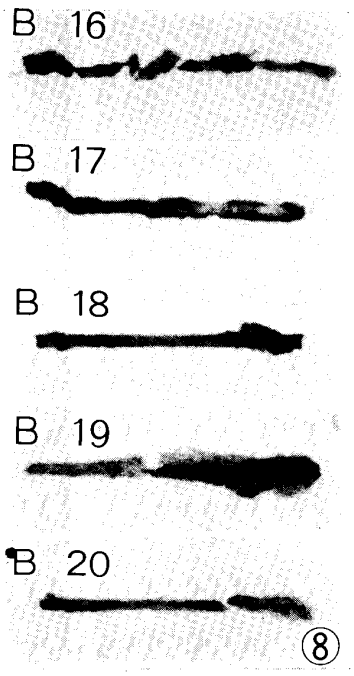
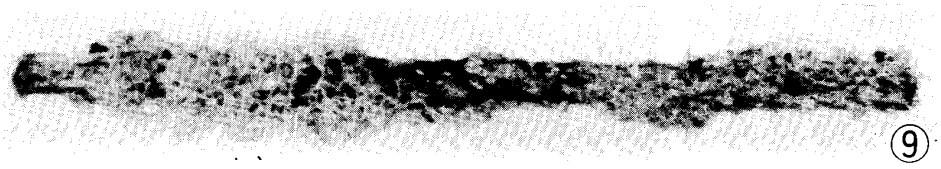
B 12
B 13
B 14
B 15
⑦


B 16
B 17
B 18
B 19
B 20
⑧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