BASIC AND PRACTICAL IMMUNOLOGIC CONCEPTS RELATED TO THE USE OF PROCESSED HETEROGENOUS BONE AND CARTILAGE IN RHINOLOGY

James Alexander Dingwall, M.D. and Robert C. Millonig, Sc.D. *

It will come as no surprise particularly in Continental centers that the subject of animal bone and cartilage for human use should be the subject of this paper. It is especially appropriate since the first report of a cross species bone graft was made by Van Meekeren in 1668 [40] who described what was an apparently successfull "graft" of fresh dog bone to a skull defect in a Russian soldier. Unfortunately for scientific posterity the heterograft was removed by Church order. Little was then documented of further transplants of heterogenous bone until Ollier's [29] masterful treatise appeared.
With the exception of experimental work at a poorly defined level, with some renewal of clinical interest, there was a hiatus of many years until the work of Orell [30] describing "os purum" and "os novum" appeared in 1937. Thereafter, and associated with the widespread interest evidenced by numerous reports about storage of homogenous bone and cartilage (banking) from the United States, there was a resurgence of interest in European centers using similar techniques applied to animal bone, especially deepfreezing, then lyophilization, and later "processing" of animal bone and cartilage. In the third, fourth and fifth decades of the 20th century the interesting work of Guilleminet, Stagnara, Dubost-Perret, the Judets and Arviset, Desbrosses, Merieux [6], Bauermeister and Maatz [24, 25] and, of course, Kingma in the Netherlands [19], appeared in the literature.
Historically, the early reports of animal cartilage implantation do not go as far back as the 17th century. Bert [3] in 1865 is generally acknowledged as the first to transplant cartilage in animals. Also, in a paper by Dupertuis [10] there was a citation of work by Middeldorf (1852). Ollier's [29] multi-volume and farseeing tract on bone of all types, and cartilage (1867) has become classical to all of us who are interested in bone and cartilage transplantation. Of particular importance to this group are the observations of Leopold [21] (1881) who was one of the first who studied fetal cartilage and concluded that even heterotransplants studied in the anterior chamber of the eye "always" grew. Also, Fischer [11] observed that fetal grafts "possessed great regenerative powers" (chondrogenic induction?). From his extensive and well controlled studies, Loeb [22] deserves great credit for his prophetic postulates about the importance of genetic predetermination as the basic cornerstone of the antigenic determinant in all tissue.

---

*) Read at the First World Conference on Rhinology, Leiden, July 9, 1963.

Clinical reports on implants of cartilage are fewer and more current than experimental studies. In 1796 Konig [20] was almost surely among the first to use cartilage transplants in man. Interest in the use of chemically preserved ox cartilage was first reported by Stout in 1933 [35]. This was fourteen years before Wardill and Swinney's work [41] stimulated serious interest by Gillies and Kristensen [21]. However, such grafts were hardly far removed from fresh heterogenous cartilage, and would have been expected to invoke an undesirable immune response.
Unknowingly, we have attempted to do what Peer said in 1956 [32] *: "The clinical use of ox cartilage by Gillies [12] and others is not criticized. Gillies has forced plastic surgeons to consider the merits of the cartilage heterograft and to produce evidence against its use. One should not ignore the possibility that future discoveries may reveal some way to render the cartilage heterograft more acceptable to human tissues."
In the same year we began a detailed study of bone and cartilage as an antigenic tissue using heterogenous (bovine) material. Our purpose was:

1. to find out, if possible, what and how many antigens were present in bone and cartilage;
2. whether they could be removed without altering the basic matrix-mineral ratio by processing; and
3. to observe such processed bone and cartilage vs. fresh in different basic laboratory studies and animal experiments in order to determine whether our processed tissue would provoke general or local immune response when used orthotopically and ectopically.

It was naturally hoped that such bone would be immunologically acceptable in man, premising early host-vascularization, rapid replacement with new host bone and eventually complete remodelling into the biologic template of the human host bone type. We did not wish to end up with "rubber" bone (EDTA treated) [33], nor did we wish an "anorganic" [15, 23] bone with practically no nitrogen content as a result of the removal from the matrix of almost all but a small fraction of the organic constituents leaving the hydroxyapatite crystals held together with a gossamer-like "something" (? remnant ground substance), having no strenght, and an unbelievable inertness which is now well known; and having neither the possibility of acting as a decent trellis, nor of evoking the slightest osteogenetic response from the host.
Most of our basic studies of animal bone and cartilage were done using careful and scientifically acknowledged immunologic techniques which were at least qualitative and to some extent quantitative.
Millonig, Amrein, and Borman [28] who explored the antigenicity of cortical bovine bone (fresh) used male albino rabbits which were immunized by weekly intramuscular injections of 100 mg. of pulverized fresh cortical bone suspended in 1 ml. of Freund's complete adjuvant which contained approximately 4 mg. of bone nitrogen. Each animal received 24 injections and 10 days following the last injection were bled, the sera collected and stored at —20° C. until used. These antisera were eventually assayed by the hemagglutination reaction according to the procedure of Boyden as modified by Fineberg [4]. Bovine serum and an equeous extract of bovine cortical bone were used as test antigens.

Following this, antibodies to bovine serum and to bovine red cell antigens were absorbed from the rabbit anti-bone serum. Antigens used for absorption were bovine serum, bovine red cell lysate, bovine albumin, gamma globulin, and fibrinogen. Absorption of antibodies was considered complete when the serum no longer gave a positive hemagglutination reaction with bovine serum or bovine red call lysate, or a precipitin reaction with bovine albumin, bovine gamma globulin, or bovine fibrinogen. The results are essentially compiled in the following table.

Table I

Antigenicity of Bovine Cortical Bone
Antigenicite de l'os bovin cortical

Demonstration, by the hemagglutination assay, of circulating antibodies in sera of rabbits immunized with bovine cortical bone.
Demonstration d'anticorps circulants en sera de lapins immunises avec de l'os bovin cortical, par l'essai hemagglutinin.

	Hemagglutination titer Test antigens		
Test sera	Bovine serum	Red Cell lysate	Cortical bone extract
Rabbit anti-cortical bone serum	1 : 128	1 : 64	1 : 32
*Idem, absorbed free of bovine serum and red cell antibodies	0	0	1 : 16

It should be noted here that rabbit anti-cortical bone serum, when absorbed free of bovine serum and red cell lysate gave a negative hemagglutination titer, but there was a positive titer when tested against a cortical bone aqueous extract. * Thus, it was felt that there was clear indication of the presence of antigens in bovine cortical bone unrelated to its serum and red cell content.
To determine the nature and number of such remaining antigens, the immuno-

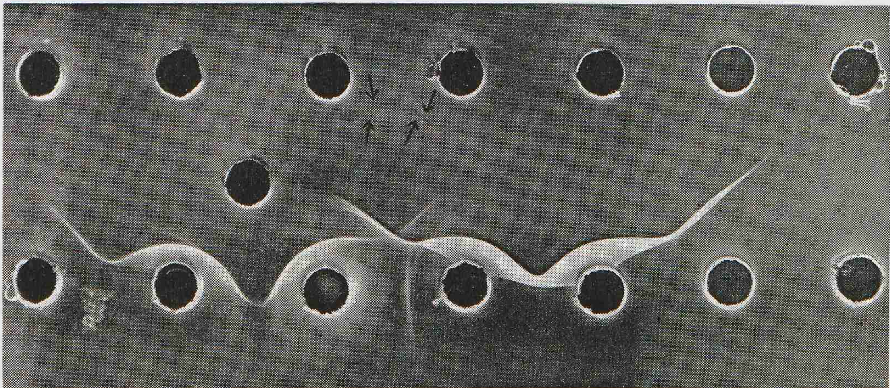

Fig. 1. Antigenecity of the aqueous extract of the test bone demonstrated by the precipitin lines (indicated by arrows).

electrophoretic method of Grabar was used (most recently described in full detail by Ouchterlony [31]. By this method it was possible to demonstrate the presence of two antigens in the aqueous extract of the test bone, separate and distinct from the serum and red cell content of the bone (Figure 1).
The precipitin lines on the plate as pointed out by the arrows show them as weak antigens which might be related to ground substance complexes, lipids, mucopolysaccharides, osteocyte protein, etc. It was thus concluded that the major antigens of fresh calf cortical bone were contained in the serum and red cell complexes.
Burwell of Leeds [5] in a Medawarian type approach implanted pieces of fresh and variously treated pieces of bone in the rabbit's ear, and used the regional nodes as an histologic indicator of the "T" [24] * (cell bound antigens) and concluded that these antibodies were produced by the antigenic components of marrow elements particularly the non-nucleated red cells, and hence were capable of producing an immune response and rejection as are the "H" (circulating) antibodies [24] *. Evidence that our processing techniques very likely get rid of most of the antigens responsible for circulating and cell fixed antibodies follows.
Briefly stated we obtain young calves and bovine embryos; **carefully detailed** controls are used in the selection. These animals are slaughtered naerby and brought immediately to our newly designed facility. Under maximum sterile precautions our bone and cartilage is obtained. It is then processed using a biologic detergent, an organic solvent, followed by a prolonged wash with sterile, filtered, deionized water; then lyophilized and sealed in vials under vacuum and stored at room temperature. Betapropiolactone is used as the sterilizing agent several times during the process.

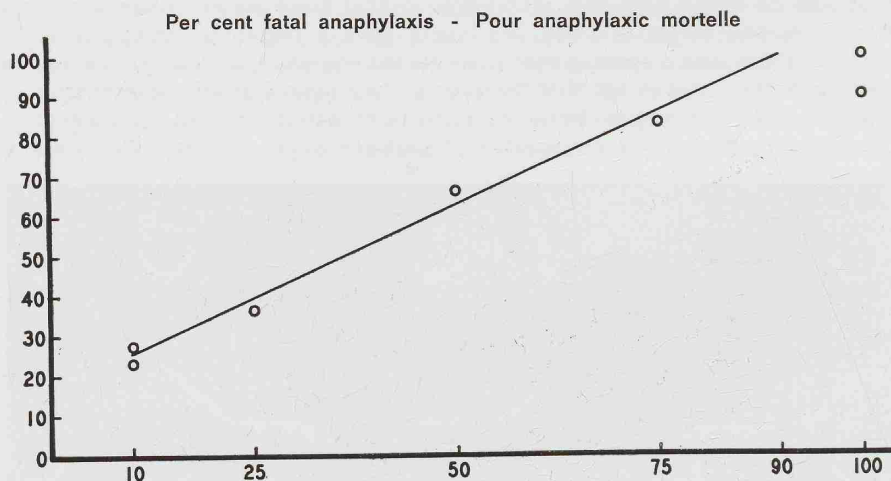

Fig. 2. Standard curve — Percent fatal anaphylaxis vs. sensitizing dose bovine serum.
Courbure étalon — pour cent d'anaphylaxie mortelle contre dose sensibilisatrice de serum bovin.
Sensitizing dose — mcg. bovine serum injected.
Dose sensibilisatrice — mcg. serum bovin injecté.

By the use of an experimentally determined curve, we found that the amount of bovine serum contained in injected pulverized bone and cartilage could be correlated with the percent occurrence of resultant fatal anaphylaxis in guinea pigs following an intravenous challenge with bovine serum. A standard curve was obtained by measuring the percent fatal anaphylaxis following an intravenous challenge dose of 1 ml. of 1 % aqueous bovine serum given to guinea pigs previously sensitized with known quantities of lyophilized whole bovine serum (Figure II).

In the actual determinations of the bovine serum content of both fresh and processed bone and cartilage, guinea pigs were well sensitized. Ten days after the last injection the animals were challenged with 1 ml. of 1 % aqueous bovine serum. Determination of the incidence of fatal anaphylaxis using the above-mentioned standard curve permitted quite an accurate quantitative expression of the bone and cartilage being tested in terms of mg. of bovine serum present per 15 gram aliquot of the bone type or cartilage under test. Our processing markedly reduced the bovine serum content of the 6 types of bone and cartilage tested [8].

Bovine serum (BS) content of fresh and processed bovine cortical, cancellous and embryo bone and cartilage.

La teneur de sérum bovin (BS) d'os bovin cortical, spongiose et embryonnair et cartilage.

Bone Sample	Type	mg BS/15 Gms Bone
Cortical	Fresh	240-390
	Processed	<5.0-20.0
Cancellous Slabs	Fresh	1300-1900
	Processed	<5.0-14.0
Ground Cancellous	Fresh	1800
	Processed	<5.0-27.5
Embryo Mandible	Fresh	900-1060
	Processed	<5.0
Embryo Orbit	Fresh	1800
	Processed	<5.0
Embryo Orbit Chips	Fresh	1800
	Processed	<5.0
Emryo Cartilage	Processed	<5.0

In another study from our laboratory, Dingwall, Millonig et al [7] felt that it would be of considerable interest to compare fresh bovine bone with Squibb processed bone in the three major parameters most often used as determinants of an immunologic response: 1) the presence, absence, or titer of circulating antibodies, 2) the presence or absence of cellbound or fixed antibodies, 3) microscopic evidence of the presence or absence of a local immune response — "heterograft reaction". For the purposes of this study, we chose guinea pigs, and in a totaal of 240 male albino animals, subcutaneous implants af cortical, cancellous, and embryo bone and cartilage, both **fresh** and **processed** were done at 10 day intervals (Figure IV).

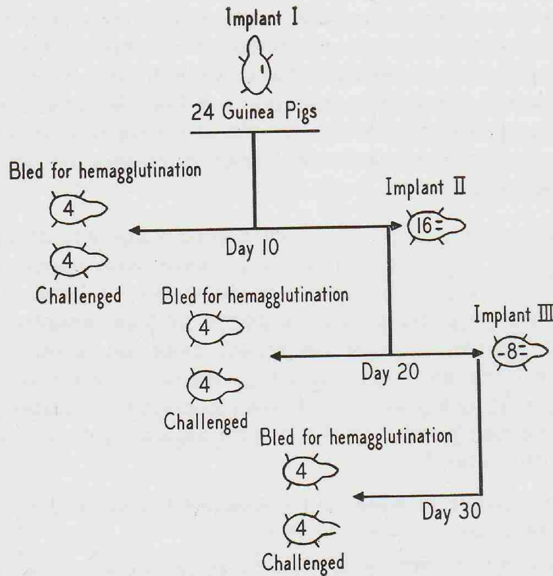

Fig. 4. Diagram of the technic of guinea pigs implants.
Diagramma de la technique d'implantations dans les tissus de cobayes.

The immunologic results are summarized in Figure V and Figure VI.

Presence or Absence of Anaphylaxis
Fatal anaphylaxis following the intravenous injection of 1 ml of 1 % bovine serum in guinea pigs 10 days following 1st, 2nd, and 3rd implants.
Présence ou absence d'anaphylaxie
Anaphylaxie mortelle par suite de l'injection intraveineuse de 1 ml de 1 % sérum bovin chez des cobays, 10 jours après les premières, deuxièmes et troisièmes implantations.

Bone Sample	Type	Fatal Anaphylaxis 1st Implant	2nd Implant	3rd Implant
Cortical	Fresh	+	+	+
	Processed	—	—	—
Cancellous Slabs	Fresh	+	+	+
	Processed	—	—	—
Ground Cancellous	Fresh	+	+	+
	Processed	—	—	+
Embryo Mandible	Fresh	—	—	+
	Processed	—	—	—
Embryo Orbit	Fresh	—	+	+
	Processed	—	—	—
Embryo Orbit Chips	Fresh	—	+	+
	Processed	—	—	—
Embryo Cartilage	Fresh	—	+	+
	Processed	—	—	—

+ = fatal anaphylaxis obtained in all four guinea pigs in group
— = no animals in groups died of anaphylaxis
+ = anaphylaxie mortelle obtenue dans tous les quatre cobayes du groupe
— = aucun animal du groupe mourut d'anaphylaxie

Hemagglutination Reaction

Circulating antibody titers to bovine serum in the sera of guinea pigs 10 days following 1st, 2nd, and 3rd implants of test bone or cartilage.

Réaction hémagglutinine

Titres d'anticorps circulants envers sérum bovin dans les séra 10 jours après les premières, deuxièmes et troisièmes implantations de l'os d'essai ou du cartilage.

Implant Sample	Type	Hemagglutination Titers		
		1st Implant	2nd Implant	3rd Implant
Cortical	Fresh	1:32	1:128	1:256
	Processed	Negative	Negative	Negative
Cancellous Slabs	Fresh	1:5	1:100	1:100
	Processed	Negative	Negative	1:64
Ground Cancellous	Fresh	1:10	x-*	1:100
	Processed	Negative	Negative	1:128
Embryo Mandible	Fresh	Negative	1:16	1:32
	Processed	Negative	Negative	Negative
Embryo Orbit	Fresh	Negative	1:2	1:8
	Processed	Negative	Negative	Negative
Embryo Orbit Chip	Fresh	Negative	1:2	1:32
	Processed	Negative	Negative	Negative
Embryo Cartilage	Fresh	Negative	1:2	1:8
	Processed	Negative	Negative	Negative

*x- not tested
*x- pas analysé

It can be seen that in every instance of a "first set" implant of all bone types and cartilage there was no evidence to suggest that either circulating or fixed tissue antibodies were evoked when the processed material was used, and low titers to embryo material developed only after the second and third implants, but even following three implants there was no fatal anaphylaxis indicating cell bound antibodies. It thus seemed reasonable to extrapolate these findings to man and conclude that no sensitization or immunization would occur, particularly since the human "antigenic dose" would be many times smaller than 250 mg. in a 250 gm. guinea pig.

Serial histologic examination of all the implants of fresh and processed bone and cartilage were carried out with particular emphasis on the group 3 implants. Unfortunately space does not permit the inclusion of all the representative photomicrographs. Suffice it to say that the histologic study was surprisingly accurate in that the "heterograft reaction" (or the lack of it) coincided most accurately with the first two parameters mentioned above when fresh vs. processed material was compared. Two photomicrographs will graphically demonstrate this.

The authors have been privileged to speak at previous meetings of the American Rhinologic Society. On these occasions as some of you will remember in was constantly emphasized that in rhinoplastic procedures the surgeon who uses implants is working in an unusual combination of an orthotopic and ectopic site wherein an implant might be bone to soft tissue, cartilage to bone, or bone to bone, etc. Therefore, as Doctor Peer pointed out in 1943 [32] *, and others after him who have used "diced cartilage" all over the body whether fresh or preserved, autogenous, homogenous or heterogenous, that vascular connective tissue followed by fibroblastic invasion, converted to a dense fibroplasia with a "collagenous cement" which binds the cartilage

pieces together is the usual result — and apparently successful. Preserved bone is anatomically, chemically and physiologically quite different than cartilage and will not survive, bathed only in a tissue milieu but requires host vascularization, replacement and eventual remodelling as an orthotopic implant. However, in the mixed tissue of the nose fibroplasia will invade and replace bone. In either instance the **host** response generally leads to a successful rhinoplastic result.

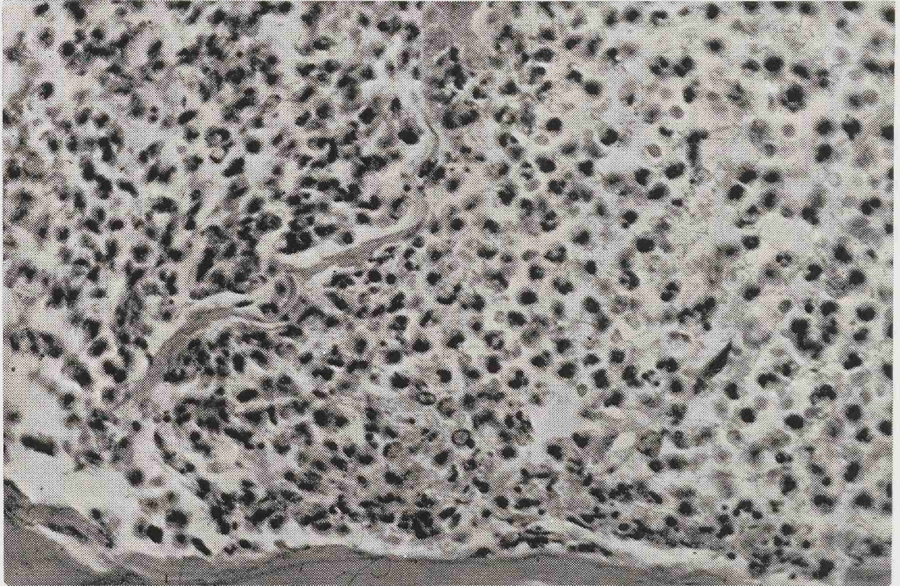

Fig. 7. Fresh cancellous bone (calf) implant (320 x).
Implantation d'os réhiculé (de veau) fraiche (320 x).

The authors do not wish to introduce axiomatic dogma into your specialty, but it does seem reasonable to say: 1) that **too little** fibrous reaction is useless; 2) **too much** inflammation (particularly on an antigenic basis) is more than likely to bring about a catastrophic clinical result (Figure VII); 3) the **right amount** of non-antigenic; non-specific inflammatory response should be ideal; and this would appear to be the value of the processed heterograft of bone or cartilage (Figure VIII).

Finally, with the superb collaboration of Doctor Maurice Cottle and members of the American Rhinologic Society more than 1500 varied rhinoplastic procedures have been done using processed bone and cartilage with a long term success rate which has been most encouraging.

As a general surgeon with orthopaedic inclinations one of the authors (JAD) wil perhaps be forgiven for mentioning that the above described processed heterologous bone has performed experimentally [1, 13, 18] and clinically [2, 16, 26, 37, 38, 39] as **well** as autogenous bone in over 1000 clinical cases with more than adequate follow-up, and as proven by ± 50 biopsies optained at intervals from 2 weeks to 19 months following grafting.

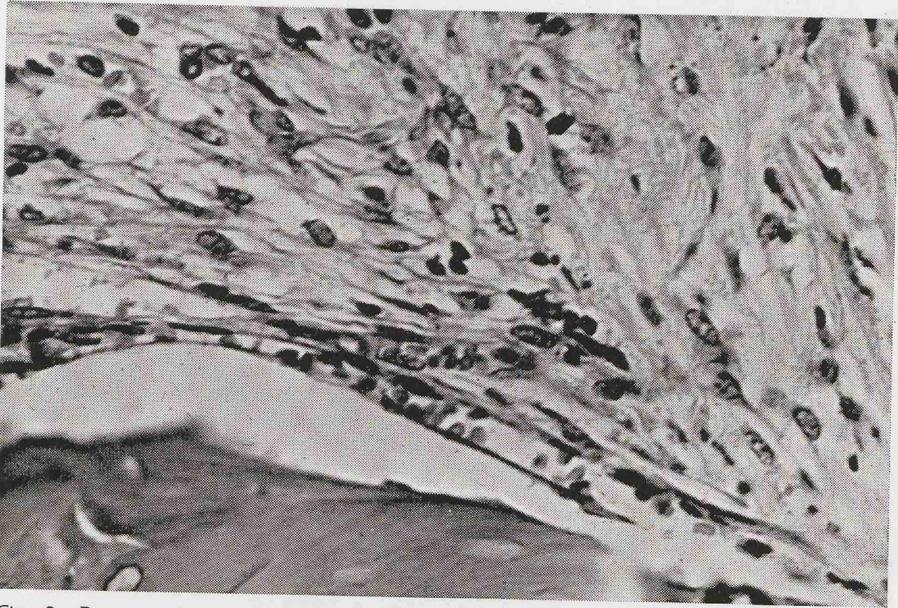

Fig. 8. Processed cancellous bone (calf) implant (320 x).
Implantation d'os réticulé (de veau) préparé (320 x).

SUMMARY

Already printed in Vol. I, no. 1.

RESUME

Déjà publié dans le Vol. I, no. 1.

REFERENCES

1. Anderson, K. J.; LeCocq, J. F.; and Mooney, J. G.: Clinical Evaluation of Processed Heterogenous Bone Transplants. Read at 26th Annual Meeting of the Western Orthopedic Association, San Francisco, October, 1962 (In Press: Clinical Orthopaedics, July, 1963).
2. Bassett, C. A. L.; and Creighton, D.: A Comparison of Host Responce to Cortical Autografts and Processed Calf Heterografts. J. Bone and Joint Surg., 44-A: 5, 842-854, July, 1962.
3. Bert, P.: Sur la greffe animale. Compt. rend Acad.sc., 51 : 587, 1865. Cited by Dupertuis, S. Milton: Actual growth of young cartilage transplants in rabbits. Arch. Surg., 43: 32, 1941.
4. Boyden, S. V.: Adsorption of Proteins on Erythrocytes Treated with Tannic Acid and Subsequent Hemagglutination by Antiprotein Sera. J. Exper. Med. 93: 107-120, 1951.
5. Burwell, R. G.; and Gowland, G.: Studies in the Transplantation of Bone. III. The Immune Responses of Lymph Nodes Draining Components of Fresh Homologous Cancellous Bone and Homologous Bone Treated by Different Methods. J. Bone and Joint Surg., 44-B: 1, 131-148, February, 1962.
6. Desbrosses, J.: Symposium sur l'os heteroplastique, Lyon, 3-4 mai, 1958. Revue de Chirurgie Orthopedique, Tome 45, No. 1, 1959, Masson et Cie- Paris.

7. Dingwall, J. A.; Millonig, R. C.: et al: An Immunologic Study of First, Second and Third Set Responses to Bovine Bone. Presented at 1962 Gordon Conference. (in press)
8. Dingwall, J. A.; Millonig, R. C.; and Westcott, W.: Reduction of the Antigenicity of Bovine Bone. Presented at 1962 Gordon Conference. (In prrss)
9. Dubost-Perret, T.: Symposium sur l'os heteroplastique, Lyon, 3-4 mai 1958. Revue de Chirurgie Orthopedique, Tome 45, No. 1, 1959, Masson et Cie, Paris.
10. Dupertuis, S., Milton-Middeldorf: Actual growth of young cartilage transplants in rabbits. Arch. Surg., 43: 32, 1941.
11. Fischer, E.: Ueber Transplantationen von organischen Materiel. Deutsche Ztschr. Chir., 17: 362, 1882.
12. Gillies, Sir Harold, and Kristensen, Harold K.: Ox Cartilage in Plastic Surgery. Brit. J. Plast. Surg., 4: 63, 1951.
13. Gresham, R. B.; and Thomas, E. D.: A Comparative Histologic Study of the Early Phases of Transplanted Bone Autografts, Freeze-dried Homografts, and Processed Calf Heterografts. Surgical Forum, Vol. XIII, American College of Surgeons, 1962.
14. Guilleminet, M.: Symposium sur l'os heteroplastique, Lyon, 3-4 mai 1958. Revue de Chirurgie Orthopedique, Tome 45, No. 1, 1959, Masson et Cie, Paris.
15. Hancox, N. M.; Owen, R. and Singleton, A.: Cross-species Grafts of deproteinised bone. J. Bone and Joint Surg., 43-B(1): 152-161, 1961.
16. Harmon, P. H.; Abel, M. S. and Dingwall, J. A.: Processed Heterogenous Bone for Anterior Interbody Spinal Fusions in the Cervical and Lumbar Regions. Scientific Exhibit, 30th Annual Meeting of the American Academy of Orthopaedic Surgeons, Miami Beach, Florida, January, 1963. (Award Certificate Winner)
17. Judet, J.; Judet, R.; and Arviset, A.: Symposium sur l'os heteroplastique, Lyon, 3-4 mai, 1958. Revue de Chirurgie Orthopedique, Tome 45, No. 1, 1959, Masson et Cie- Paris.
18. Karges, D. E.; Anderson, K. J.; Dingwall, J. A.; and Jowsey, J.: Experimental Evaluation of Processed Heterogenous Bone Transplants. Read at 26th Annual Meeting of the Western Orthopedic Association, San Francisco, October, 1962. (In press: Clinical Orthopaedics, July, 1963)
19. Kingma, M. J.: Results of Transplantation with Preserved Calf Bone. Arch .Chirurg. Neerl. 12(3): 221-235, 1960.
20. Konig, F.: Reaction of Cartilage to Injury. Arch. Klin. Chir., 124: 1, 1923; Abstr. J. A. M. A., 81: 1646, 1923.
21. Leopold, G.: Experimentelle Untersuchungen über die Aetiologie der Geschwulste. Virchows Arch. Path. Anat. 85: 283, 1881.
22. Loeb, L.: Transplantation and Individuality. Physiol. Rev., 10: 547, 1930.
— idem: The Biological Basis of Individuality, Springfield, C. C. Thomas, 1945.
23. Losee, F. L., and Hurley, L. A.: Successful Cross-Species Bone Grafting Accomplished by Removal of the DonorOrganic Matrix. Naval Med. Res. Inst. 14: 911-948, 1956.
24. Maatz, R. and Bauermeister, A.: A Method of Bone Maceration. J. Bone and Joint Surg., 39-A: 153-156, 1957.
24* Medawar, P. B. (1959a): Iso-antigens. In Biological Problems of Grafting. Edited by F. Albert and P. B. Medawar: Blackwell Scientific Publications.
— idem: (1959b): In Cellular and humeral Aspects of the Hypersensitive States. Edited by H. S. Lawrence. London. Cassel.
25. Maatz, R.: Clinical Tests with Protein-Free Heterogenous Bone Chips. Bull. Soc. Int. Chir., 19: 607-611, 1960.
26. Merieux, M.: Syposium l'os heteroplastique, Lyon, 3-4 mai, 1958. Rveue de Chirurgie Orthopedique, Tome 45, No. 1, 1959, Masson et Cie- Paris.
27. Meyer, B. S., Norwood Clinic, Birmingham, Alabama, U.S.A., Personal communication.
28. Millonig, R. C.; Amrein, B.; and Borman, A.: Antigenicity of Bovine Cortical Bone. Proc. Soc. Exptl. Biol. Med. 109: 562-564, March, 1962.
29. Ollier, L.: Traite experimental et clinique de la regeneration des os et de la production artificielle du tissu osseux, vol. 1, p. 162. Paris, V. Masson & fils, 1867.
— idem: Traite des Resections. Masson et fils, 1885.

30. Orell, Svante: Experimentalchirurgische Studie über Knochentransplantate und ihre Anwendung in der praktischen Chirurgie. (Vorläufige Mitteilung.) Deutsche Zeitschr. F. Chir., 232: 873-885, 1937.
— idem: The use of os purum in Bone Implantations, Surg. Gynecol. and Obstet. 66: 23-36, 1938.
31. Ouchterlony, O.: Diffusion in Gel Methods for Immunological Analysis. Progress in Allergy, 16: 30-346, 1962.
32. Peer, L.: Diced Cartilage Grafts. Arch. Otolarygn., 38: 156, 1943.
32* Peer, L.: Transplantation of Tissues. Vol. I (Williams and Wilkins, Baltimore 1955).
33. Ray, R. D.; Bone Implants. A Preliminary Study. J. Bone and Joint Surg., (39-A(5), 1119, 1957.
34. Stagnara, P.: Pre-Rapport. Apropos des Heterograftes. Symposium sur l'os heteroplastique, Lyon, 3-4 mai, 1958. Revue de Chirurgie Orthopedique, Tome 45, No. 1, 1959, Masson et Cie- Paris.
35. Stout, P. S.: Bovine Cartilage in Correction of Nasal Deformities. Laryngoscope, 43: 976, 1933.
36. Urist, M. R.; Mazet, R.; and Bechtol, C. O.: Senile Osteoporosis as a Disorder Influencing Treatment and End Results of Fractures of the Hip: With a Preliminary Report on the Use of Collapatite. American Surgeon, 25: 883-890, November, 1959.
37. Urist, M. R.: Bone: Transplants, Implants, Derivatives, and Substitutes — A Survey of Research of the Past Decade. A.A.O.S. Instructional Course Lectures XVII: 184-195, 1960.
38. Urist, M. R.; and Moon, N. F.: Fractures of the Hip in Patients with Severe Osteoporosis: The Effect of Collapatite Pegs on the Process of Healing. The Journal of Trauma, 1: 373-391, 1961.
39. Urist, M. R.: Processed Cortical Bone for Internal Fixation in Lumbosacral Arthrodesis Acta Orthopaedica Scandinavica: 32, 1962.
40. Van Meekeren, J. J.: Heel- en geneeskonstige aanmerkingen. Amsterdam, C. Commelijn, 1668.
41. Wardill, W. E. M., and Swinney, John: Bovine Cartilage in Plastic Surgery. Lancet, 2: 389, 1947.

James Alexander Dingwall M.D.
c/o Squibb Institute for Medical Research
745 Fifth Avenue,
New York 22, N.Y., U.S.A.