THE EFFECTS OF SURGICAL EXCLUSION OF AIR FROM PNEUMATISED BONE

with

a preliminary study of the general and intra-osseous vasculature of the wing of the domestic fowl.

by

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A thesis submitted for the Degree of Master of Surgery, University of Adelaide.

1965.

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SUMMARY.

During the past 40 years, the surgery of the pneumatic system of the human temporal bone has undergone a considerable change. Although cavitation surgery still has an important place, numerous obliterative procedures have been developed which employ muscle, fat, bone, cartilage or even foreign substances such as methyl methacrylate in order to fill the operation cavity. The local changes which these materials undergo have been widely studied, but no systematic investigation has been undertaken to determine what happens to normal developing and fully developed air cells of the mastoid type when they have been isolated from the atmosphere as a result of the performance of such obliterative procedures.

In order to investigate this problem, the pneumatised humerus of the domestic fowl, Gallus domesticus, was chosen for the experimental investigation. The events occurring during the pneumatisation process in this bone, and the histological features of the air space lining, are identical with those seen in the human temporal bone. In addition, because of the large size of its pneumatic system and the relatively complex trabecular arrangement, the bone was found to be more satisfactory for the purpose of the experiment than the Bulla mastoidea of animals such as the cat and guinea pig. Only cockerels were used. Hens were excluded from the investigation because of the peculiar physiological medullary new bone formation which occurs in laying birds. It was considered that this phenomenon, although easily recognisable histologically, might unnecessarily complicate the interpretation of the results.

A preliminary study of the arterial supply of the wing of the domestic fowl was first carried out, not only to determine the vascular anatomy in the region of the upper end of the humerus, but also to establish the origin and course of the vessels supplying the humerus with blood. The intraosseous vascular pattern of the pneumatising and fully pneumatised humerus was then examined and compared with that of the ulna in order to establish the modification of the vascular pattern in association with pneumatisation, and to assess whether the proposed operative procedures would seriously interfere with the blood supply of the bone.

The birds were next arranged in two series on the basis of a radiological assessment of the stage of pneumatisation. The first series of 24 birds showed full pneumatisation, the second series of 20 birds showed partial pneumatisation. In one subgroup of each series the foramen pneumaticum was blocked with a pedicled muscle graft. In the other subgroup of each series the foramen pneumaticum was blocked with methyl methacrylate. The operations were performed on the left humerus, the right being used as a control. Serial sacrifice was carried out weekly over 4 weeks, and monthly over a period varying from 5-8 months. All bones were first inspected macroscopically and then examined histologically. In addition, perfusion studies were carried out on several of the post-operative humeri in order to determine the vascular changes within the pneumatic system following surgical exclusion of air, and to confirm that the operative procedures did not seriously

interfere with the blood supply of the bones.

As a result of the operative procedures and the consequent exclusion of air from the pneumatic system of the humerus, changes were seen not only in the epithelium lining the air space, but also in the sub-epithelial tissues, the bone substance itself, and the contents and size of the pneumatic system. These changes occurred in a definite time sequence.

Pneumatisation ceased, and congestion and dilatation occurred in relation to the vessels of the subepithelial tissues. In association with this alteration in vascular dynamics, the subepithelial tissues became oedematous, and the air space became filled with a fluid transudate. The previously flat lining cells became markedly round in appearance, and underwent a foamy cytoplasmic change. They were found to be heavily laden with fat, and many of them floated off into the fluid-filled air space.

The subepithelial mesenchymal tissue then began to proliferate out into the air space, being rapidly followed by a vascular outgrowth which provided a blood vessel core for the developing tissue. In some islands within this tissue, numbers of stellate mesenchymal cells became round in appearance and underwent a foamy change due to the intracellular deposition of lipoid material. In other areas, a transformation into true fat cells was seen taking place in relation to the ramifying blood vessels. Myeloid cells appeared in increasing numbers in association with this change, but the haemopoietic activity was short-lived. The

free myeloid elements soon decreased as the fatty tissue matured, and as the vascularity became reduced. The overall change appeared to represent the process of reconversion of the bone to a marrow-containing one.

Two or three months after operation, masses of cholesterol granuloma tissue were found scattered throughout the pneumatic system. The connective tissue of the granulomata contained numerous multinucleate giant cells and foam cells heavily laden with fat. No haemosiderin granules were seen, and surprisingly little collagen was present. In many sections it could be seen that the granulomata were formed as a reaction to crystals deposited in the tissues themselves following focal areas of degeneration in the mesenchyme. In other sections, there was no doubt that many of the granulomata were formed by the proliferation of strands of connective tissue cut into pneumatic spaces filled with cholesterol crystals and fat embedded in a protein gel.

Four types of new bone formation were also observed:-

a. new bone was deposited on the existing trabeculae, so thickening and strengthening them,

b. bony trabeculae and plates were laid down in an orderly fashion just beneath the lining epithelium,

c. isolated islands of new bone appeared in the midst of the masses of vascular mesenchyme. These enlarged and fused with adjacent islands giving rise to a bizarre reticular pattern,

d. a deposition of new bone was observed in the cholesterol

granulomata.

These progressive space-filling and obliterative changes reduced the original air space to a scattered collection of small fluid-filled spaces enclosed in a mass of newly formed tissue. The changes were correlated with the development of a negative pressure, with a rising protein level in the fluid transudate, with the accumulation of cholesterol crystals in the fluid filling the obstructed pneumatic system, and with local alterations in the intraosseous vasculature. The development of the changes was also correlated with the degree of pneumatisation at the time of operation, and with the nature of the operative method of obstruction of the pathway of ventilation of the bone.

The relevant historical and scientific background of the problem has been reviewed.

ACKNOWLEDGEMENTS.

I would like to express my indebtedness to Mr. Ronald Macbeth, Director of the Department of Otolaryngology, Radcliffe Infirmary, Oxford, for his assistance in selecting the subject of the present work. Without his help and encouragement the investigation would never have commenced.

In addition, I would like to take the opportunity of expressing my gratitude to Professor J. Trueta for allowing me full use of the facilities of the Nuffield Department of Orthopaedic Surgery, Oxford.

In this regard, my thanks are due to Miss Maureen Litchfield for her kindness and painstaking care in the preparation of the histological and Spalteholz specimens; to Miss Gillian Stokes for taking the plain and fine grain radiographs; and to Mr. A. Timms for his care of the animals and his assistance with anaesthesia during the surgical procedures. The numerous microphotographs were taken by Dr. Kitty Little, Lecturer in Research at the Nuffield Orthopaedic Centre, and I am indebted to her for valuable advice during the performance and writing-up of the present work. I am also grateful to Mr. David Drury and the staff of the Department of Medical Photography at the Nuffield Orthopaedic Centre for preparing the final photographs which illustrate this thesis.

The biochemical estimations were kindly conducted by Dr. David Griffith of the Department of Biochemistry, University of Oxford.

The "Sevriton Simplified" acrylic material was supplied by the Amalgamated Dental Co., Ltd., London.

Finally, my thanks are due to the Nuffield Committee for the

advancement of medicine for electing me to the position of Surgical Research Assistant in the Division of Surgery in the University of Oxford Medical School. It was during the twelve month tenure of this post that the major part of the present investigation was carried out.

The present work does not contain any material which has been submitted or accepted for the award of any other degree in any University.

I. INTRODUCTION.

1. The Nature of the Problem Investigated.

The pneumatic cells of the human temporal bone are normally in direct anatomical communication with atmospheric air via the tympanic cavity and Eustachian tube. In certain circumstances this communication may become pathologically obstructed. If a group of air cells becomes the subject of an inflammatory process, congestion and swelling of the mucosa together with the formation of granulation tissue, scar tissue or even new bone may lead to their isolation from the atmosphere. Further, if obstruction of the Eustachian tube develops for any reason, a total isolation of the whole pneumatic system results.

Surgical procedures involving the air cell system may also result in the isolation of groups of cells. The extent of this depends on the nature of the procedure used. When a mastoid cavity is allowed to epithelialize, is covered by a graft, or is obliterated by various means, it must be recognised that residual air cells will be isolated by obstruction of their normal communication with the atmosphere.

2. The Subject of the Present Investigation.

Medical history abounds with arguments against almost every new advance in theory and practice. The history of mastoid surgery is no exception, especially where the use of obliterative techniques is concerned. Among the valid objections that have been raised, are the dangers of burying irreversible inflammatory disease and of burying pockets of squamous epithelium. In addition, however, it has been

suggested that there exist dangers of interfering with a supposed respiratory function of the lining membrane of the mastoid alveoli (Adams 1961), of continued secretion from the buried mucosa (Walsh 1958) and of possible cyst formation (Guilford, Wright and Draper 1958). Virtually no experimental work has been carried out to ascertain whether these suggestions are valid, and it is the purpose of this work to shed some light on what in fact does happen to normal air cells as a result of surgical measures directed towards their isolation from the atmosphere.

3. The Animal used in the Investigation.

In the case of the animals commonly used for otological research, such as the guinea-pig and cat, the bulla mastoidea is usually a single-celled space with perhaps a small number of additional recesses. This type of air-cell system was not considered sufficiently large or complex for the purposes of the present study. The multi-cellular system of the temporal bone of animals such as the monkey, ape, gorilla, crocodile or kangaroo (Tumarkin 1957) could have been used, but available finance and facilities prevented this.

It is known that the pneumatic system of the humerus of the male domestic fowl resembles that of the human temporal bone both in structure and development, and that it is relatively large and complex. For this reason it was felt that there were good grounds for applying observations from experiments on this bone to conditions in the human temporal bone. The humerus of the male domestic fowl (Gallus domesticus) was therefore chosen for the experiments.

4. The Plan of the Main Study.

No previous worker has carried out a planned investigation into the histological and vascular changes occurring in developing and fully developed normal, healthy pneumatic cells following their isolation from the atmosphere by measures similar to those employed in otological surgery. It was therefore decided to obliterate the foramen pneumaticum of the humeri of both young cockerels and mature cocks by using two methods, the muscle graft and methyl methacrylate, both of which are in current use in obliterative mastoid surgery.

The experimental series was planned to extend over a six to eight month period so that progressive information could be obtained at serially predetermined times of sacrifice. It was also thought that by working with both immature and well-developed animals, additional knowledge could be acquired regarding the effects of obliterative measures on pneumatic systems which had reached different stages of development.

5. The Purpose of the Preliminary Vascular Studies.

It was realized that the performance of experimental procedures involving bones, and the interpretation of resultant pathological changes presupposed a detailed knowledge of the general anatomy of the part, the blood supply of the bone itself, and, in the case of the chick humerus, a knowledge of normal histological and vascular changes of pneumatisation. An extensive review of the literature revealed that although previous workers had described the general anatomy of the avian wing and had

dealt with the normal features of avian bones including the histological changes of pneumatisation, no adequately detailed information was available concerning -

(i) The arterial anatomy of the wing of Gallus domesticus proximal to the carpus,

(ii) The intra-osseous blood vessels of the marrow containing bones of the wing of Gallus domesticus,

(iii) The alteration in the pattern of the interosseous vessels as a result of pneumatisation.

Because the operative procedures and the interpretation of the histological material of the main study depended on a knowledge of these points, it was obvious that preliminary vascular studies were also needed for the present work.

II. HISTORICAL BACKGROUND.

The historical background of the problem under consideration goes back to the very beginning of temporal bone surgery. Many excellent accounts of the early events have been written (Whiting 1905, Ballance 1919, Kerrison 1930, Mollison 1930, Sonneschein 1936, Kopetzky 1938, Guthrie 1940, Stevenson and Guthrie 1949, Shambaugh Jr. 1959) and therefore only a brief outline will be given here. However, the more recent phase concerned with the various obliterative methods has not received such attention and will be dealt with in more detail. It is largely the use of these techniques which has led to the need for the present investigation.

1. Period of Experimentation.

Galen was evidently aware of the existence of mastoid surgery, as he is recorded as saying that carious bone should be removed after making an incision behind the ear (Guthrie 1940). Later, Ambroise Pare (1510-1590) is said to have wished to operate on the skull of Francois II of France to drain away pus when the King had a discharging ear, but apparently he was not permitted to perform the operation (Kemble 1936).

The next thought was that trephination of the mastoid process might relieve deafness. Johannes Riolanus probably first propounded this in 1649 (Mollison 1930), and in 1656, Rolfink, apparently independently, made a similar suggestion (Whiting 1905). The theory was to establish an artificial vent which would provide an avenue of escape for distressing and tumultuous noises. There is no record, however, that either of

these men actually put his theory into practice.

Some time afterwards, Valsalva, in 1740, and later Heuermann, in 1757, each reported a case of spontaneous perforation of the mastoid cortex as a result of inflammatory necrosis (Whiting 1905). They practised syringing through the perforation and recorded the advantages of utilizing the opening. However, they did not appear impressed with the possibility of making an artificial opening for the relief of chronic suppuration of the ear, and it was Jean Louis Petit who was the first to appreciate its value. In about 1740, he performed the first recorded successful operation on the mastoid for the evacuation of pus, and wrote that he considered purulent inflammation of the bone, with caries, was the only indication for trephination of the process (Petit 1774). In 1776, Jasser, a Prussian military surgeon, also opened the mastoid in a recruit suffering from earache, ear discharge and deafness. His method was to bore a hole in the bone and to syringe through the artificial opening, thereby driving out the pus (Hollison 1930). The so-called "Jasser operation" was speedily recognized and widely discussed, but, perhaps because Jasser was convinced of the value of the operation as a remedial agency in deafness, attention was directed more and more to its use for the relief of deafness and less and less to the purpose as outlined by Petit.

In about 1790, Fielitz claimed to have successfully performed the operation on three deaf patients with complete restoration of hearing. However, his results cannot be relied upon (Whiting 1905), and it is no

wonder that subsequent operators failed to repeat his success. A.F. Loefler, a Prussian surgeon, undertook the operation for the relief of deafness at about the same time as Fielitz, but failed to get results (Whiting 1905). Hagstrom, a Swedish physician, also failed to benefit his patients (Whiting 1905).

The procedure fell further into disrepute when, in 1791, the Danish court Physician, Baron von Berger, requested such an operation for the relief of his own tinnitus and deafness. Alexander Kölpin (1731-1801) performed this service for him in Copenhagen, but the Baron died twelve days later from purulent meningitis (Stevenson and Guthrie 1949). The following year, Proet, also in Copenhagen, undertook the "Jasser operation" for deafness, but the patient developed a secondary infection in his ear and was lucky to escape with his life (Whiting 1905).

After these unfortunate results, the operation was virtually abandoned for almost a century.

2. The Reawakening - the development of the simple operation.

In 1853, Sir William Wilde (1815-1876) of Dublin published his book on "Aural Surgery" and suggested that "should the mastoid process become engorged or even an indistinct sense of fluctuation be discovered, we should not hesitate to make a free incision at least one inch in length .... until the point reaches the bone, to secure complete division of the periosteum" (Wilde 1853). He advocated the incision as a measure for the relief of periostitis incident to mastoiditis, but in fact its

performance was senseless in that it did not reach the source of the disease. Its only logical value was in cases where the inflammatory process had already perforated the outer table of the bone.

Perhaps it was because of this that Joseph Toynbee (1815-1866), who was a more astute pathologist than Wilde, wrote that "perforation of the mastoid process suggests itself in serious cases likely to terminate in death. I have never performed the operation but should not scruple to do so where the life of the patient was threatened" (Toynbee 1860). In a short time the operation was being performed in many parts of the world. In 1860, the French surgeon, Forget, reported success after operating on a young man with mastoid disease (Forget 1860). A year later, Anton von Tröltsch (1829-1890) published a paper describing a successful operation which he had performed in 1858 on a young girl of sixteen (von Tröltsch 1861). Another year later, Lawrence Turnbull (1821-1900) performed the operation for the first time in America (Turnbull 1862). In 1864, Ludwig Mayer performed the operation in Germany (Whiting 1905), and, in 1868, James Hinton (1822-1875), the first Aural surgeon to Guy's Hospital, became the first to open the antrum and cells in England (Hinton 1874).

Despite this activity, the procedure was still by no means common, only thirty-five operations being recorded in the literature up to 1873 (Buck 1880). Whiting, quoting from Roosa's book of 1885, described the accepted way of performing the operation up to that time. The bone was trephined by working "in a direction inward, forward and upward", but no

positive directions were given as to the depth to which the instrument should go. As a result, it was not uncommon for the antrum not to be entered and the lateral sinus to be struck by mistake (Whiting 1905).

It is to Hermann Schwartze (1837-1910), in 1873, that we are indebted for the first clear account of the indications for the simple mastoid operation and the technical principles involved (Schwartze and Eysell 1873). The "Schwartze operation", however, did not include the removal of the mastoid tip, and it was E. Gruening, of New York, who was probably the first to advise this as a matter of routine (Whiting 1905). Finally, it is perhaps to Frederick Whiting that credit is due for focussing attention on the importance of systematic thoroughness in the performance of the operation, and the "removal of the pneumatic spaces and diploic cells at the posterior root of the zygoma" (Whiting 1905).

3. The Period of Development - the evolution of the radical operation.

The indications which Schwartze set out for guidance in the performance of his operation were comprehensive, perhaps too comprehensive. The last of them concerns cases of "tedious otorrhoea ..... not sufficiently accounted for by the conditions of the Eustachian tube and tympanum ..... the discharge being offensive and mixed with cholesteatomatous masses". It is not surprising, therefore, that modifications of the Schwartze operation were suggested to deal more adequately with these cases.

In 1873, Anton von Tröltsch suggested a variation of the simple operation which included many of the essentials of the present "radical"

operation (von Tröltsch 1873), but it was E. von Bergmann (1836-1907), in 1889, who was probably the first to use this name for operations that included removal of the posterior and superior osseous meatal walls (von Bergmann 1889). In the same year, Ernst Küster (1838-1930) claimed that Schwartze had never adequately penetrated beyond the antrum and thus he recommended a free opening of the bone with removal of the posterior osseous wall (Küster 1889). Ludwig Stacke of Erfurt (1859-1918), also in 1889, wrote of his operation to open the tympanum, attic and antrum into one cavity by chiselling away the inner extremity of the roof and posterosuperior wall of the bony meatus. He then progressed from within outwards until the attic and antrum were widely exposed (Stacke 1889). In 1890, Emanuel Zaufal (1837-1910) felt it less dangerous to proceed from without inwards. He first removed the mastoid cortex and posterosuperior canal wall and then progressed slowly inwards until only the thin, narrow, inner margin of the posterosuperior canal wall remained. This was then gently removed (Zaufal 1890).

Thus, just as the simple mastoid operation had become known as the Schwartze operation, the radical operation was later to be known as the Zaufal or Stacke operation and involved removal of the superior and posterior osseous meatal wall, and all remnants of malleus, incus and tympanic membrane.

4. The Period of Modification. - the development of modified radical operations.

Just as the Schwartze operation had been considered inadequate to

deal with certain cases, so the Zaufal or Stacke operation began to be considered as too destructive to warrant its use in many circumstances. Thus, in 1893, A. Jansen described "the radical operation with conservation of the ossicles and drum membrane, as well as the removal of the outer attic wall and the exposure of the posterior and inferior tympanic cavities". His work was apparently not widely known, and led him to say quite hotly, in 1908, that "the radical operation without the removal of the ossicles is my discovery" (Jansen 1908).

Others were also becoming less radical, and, in 1897, even Stacke himself was beginning to advise conservative operations (Mollison 1930). Two years later, O. Körner suggested that in certain circumstances an attempt should be made to leave the tympanic membrane and ossicles in place (Korner 1899). In 1906, W. S. Bryant wrote of his own attempt to modify the radical mastoid operation to allow the preservation of hearing (Bryant 1906 a), and in the same year, C. J. Heath described an operation in which he partially removed the posterior osseous wall and left the ossicles, tympanic membrane and outer attic wall (Heath 1906). In effect, this opened the antrum into the meatus in accord with his theory that the antrum was the key to the problem of the persistence of the disease. The procedure did not meet with general approval, however, as it was felt that the indications were extremely limited. In 1908, W. L. Ballenger lectured on his own "meato-mastoid operation" and clearly set out the indications and contra-indications for the procedure (Ballenger 1908). His lecture was subjected to heated discussion,

however, and C. F. Welty claimed that his technique was no different from that of Heath (Welty 1908).

By this time, many workers were performing modified radical operations and, in 1910, G. Bondy, of Vienna, carefully set forth clear indications for an operation involving the removal of the outer attic wall and the taking down of the bridge. In this way the attic and antrum were exteriorized while leaving the tympanic membrane and ossicles intact (Bondy 1910). Although the procedure was similar to that which Jansen had used 27 years earlier, it has come to be known as the Bondy modified radical mastoidectomy (Shambaugh Jr. 1959).

5. Period of elaboration - the destandardization of mastoid surgery and the development of obliterative methods.

The basic techniques and principles of mastoid surgery had thus become established, but, before long, it was gradually acknowledged that the exclusive use of three standard operations was not sufficient. Workers over the past fifty years have therefore elaborated a multiplicity of variations having closer regard to the nature and extent of the disease present, and to the preservation or restoration of hearing.

It was also realised that the end-results of "cavity surgery" had not been satisfactory. Difficulty had been experienced with securing healing of the cavities, and, even after they had become dry and healed, many were seen to break down and discharge again. Strenuous efforts were therefore made to get the cavity healed, and to provide it with an intact healthy lining.

The first method of obtaining healing of simple mastoid cavities was based on the belief that all pus containing cavities should be treated as open wounds and compelled to fill up with granulation tissue from the bottom (Reik 1906). A preliminary flushing of the cavity with an antiseptic solution was carried out, followed by firm packing with antiseptic gauze. Frequent redressing and repacking was performed, and occasionally there was a need to persist with this for up to six months.

The next step came in 1886, when C. J. Blake, of Boston, began to use the "blood-clot dressing". After careful removal of all disease, he allowed the cavity to fill with blood and then performed a primary suture of the wound, except for the lower end which he "left open to allow for seepage" (Blake 1906). W. S. Bryant also tried this method with success (Bryant 1906 b), and H. O. Reik subsequently outlined the logic of using a blood clot because of "its bactericidal power, and its provision of a fibrinous framework" through which fibrous tissue could grow (Reik 1906).

Meanwhile, the radical operation had been developed, and the first attempts were being made to secure an epithelial lining for the cavity. Pedicled meatal skin flaps were used on the principle that the squamous epithelium of the flap would proliferate and line the remainder of the cavity. Stacke described his own type of flap in 1893 (Stacke 1893), and this was rapidly followed by many modifications, such as those of Panse (1893), Jansen (1893), and Körner (1899).

As the healing of cavities came to be a problem, however, it was soon felt that it would be advantageous to provide an immediate skin covering for the whole bony surface. Hollinger states that in 1891 he assisted when the first Thiersch graft was placed in a radical mastoid cavity, but does not state who performed the operation (Hollinger 1908). However, Siebenmann was utilizing primary skin grafting by 1893 (Siebenmann 1893) and, since then, the method has been used by innumerable workers with minor variations. In 1949, Lempert described the use of free meatal skin (Lempert 1949), House (1949) used very thin full thickness skin from the postauricular area and Farrior (1949) utilized split skin from the thigh. In 1955, Campbell described his use of split thickness grafts from the inner surface of the upper arm (Campbell 1955). The principle, in all cases, was to get more prompt healing and to prevent the filling-in of the cavity by granulation tissue (Stewart & Fraser 1930).

However, in 1930, Kerrison felt that skin grafting at the time of the primary operation was not advisable, and advocated delayed skin grafting after 10-14 days when the cavity had become lined with a layer of healthy granulation tissue (Kerrison 1930). Guilford and Wright again described this concept in 1954 and claimed to be the first to use the method in fenestration cavities (Guilford and Wright 1954).

Despite these efforts, many cavities were still not satisfactory, even though great care was being taken to eradicate all diseased air cells. It was next considered that non-healing and breakdown were due to the

fact that skin grafts placed directly in the bony cavity had only a very meagre blood supply. As a result of this concept, the large, pedicled skin flap was developed so that the lining skin might bring its own blood supply with it. Mosher, in 1911, appears to have been one of the first to use the method, and took a flap of skin from over the mastoid process (Mosher 1911). Since then, many others have used this type of flap including Khan (1927), Vicencio (1956), Charland (1960), Gundersen (1961) and Grahme (1963). In 1959, Beales and Hynes utilized the same principle, but took a flap from the cranial surface of the pinna (Beales 1959, Hynes 1959).

However, other problems soon arose with the pedicle method because of the presence of hair follicles and sweat glands in the flap, and the fact that the cavity was lined with skin without the surface migratory properties with which normal endomeatal skin is endowed. Products of desquamation, therefore, tended to accumulate and required continued attention, and patients still had to be forbidden from activities which allowed water to enter the ears. Interest was thus diverted to the possibility of obliterating the post-operative cavity partially or completely, the advantages being more rapid healing, decreased area of squamous epithelial surface and, therefore, decreased desquamation and accumulation of debris plus decreased graft dermatitis and post-operative suppuration (Guilford 1961), decreased need for further post-operative care (Fritz and Crawford 1960), and increased protection of vital structures, such as the facial nerve, dura mater and lateral sinus,

which may have been exposed.

Perhaps the first step towards this goal was the intelligent use of blood clot. In 1949, Daggett described his use of it in the operative treatment of chronic suppurative otitis media. He preserved the meatal skin and soft tissues and allowed the clot to fill the posterior part of the cavity. The clot subsequently organised and pulled the canal back from behind, opening it up (Daggett 1949). House, also in 1949, mentioned that even if he was forced to discard the posterior meatal skin, he was still able to allow the cavity to fill with blood and then heal by fibrosis. He claimed that the end result was an obliteration of the cavity (House 1949). McGuckin has also found this method satisfactory, and uses a small meatal pack to secure the conditions required to allow blood to accumulate and clot in the cavity (McGuckin 1964). The method is an old one, however,

It was felt, by others, that the blood clot method was somewhat uncertain, and, before long, normal body tissues were being utilised to facilitate the obliteration. In 1928, H. Kisch described his use of both free and pedicled temporal muscle to obliterate the cavity left at radical or partial radical operation (Kisch 1928) and D. Guthrie (1928) also reported good results. Two years later, Jenkins (1930) claimed he had been using the principle for three to four years, and W. A. Hill (1930) reported several other cases. Despite the fact that L. G. Brown (1930) stated that muscle grafts should be limited to cases of permanent antral fistula, deep depressions, or cases of a large bony

removal, Meurman, by 1938, had started to use a sternomastoid flap to overcome the unfavourable size and shape of the operation cavity (Meurman and Ojala 1949). In reporting further cases, Meurman and Ojala (1949) stated that they realized that by filling the cavity with such a flap, remaining pneumatic cells were shut off. They felt, however, that this did not give rise to complications provided that the cells were healthy. The use of muscle grafts was then extended by Rambo, in 1958, when he used such a flap to cover both the cavity and middle ear space, placing the meatal skin back on this (Rambo 1958). In 1960, Fitz, a disciple of Rambo, reported complete healing in four weeks with little need for further post-operative care using this method (Fritz and Crawford 1960). The literature now abounds with reports of success with myoplasty procedures, and Thorburn (1960, 1961), Guilford (1961), Peck (1961), Palva (1963) and Witcher and Streit (1963) are only a few of those who have written about them.

Muscle is by no means the only tissue that has been used, however, and fat grafts were being tried at about the same time as muscle flaps were being utilized. D. Guthrie (1928) spoke of the use of fat grafts to fill the mastoid cavity, and, in 1930, Brown also mentioned their use (Brown 1930). Two years later, Straatsma and Peer wrote of the repair of postauricular fistulae by means of free fat grafting (Straatsma and Peer 1932), and Bennett reported similar success a year later (Bennett 1933). Before long, other workers were trying the method, and reports were published by Suarez (1949), Rodriguez (1952) and Van Deinse and

Van den Borg (1952) to mention but a few.

However, it is known that the cells in fat and muscle grafts depend to a large extent on having an adequate blood supply if they are to survive transplantation. In fact, it is probable that only when autogenous muscle flaps are transplanted with an intact blood supply and motor innervation, and are not subject to disuse, do they remain as such. Certainly, free autogenous muscle grafts always degenerate and lose their structure as muscle, and it is also thought that in free fat grafts, only the more hardy and advantageously located fat cells can survive. In both cases, however, the connective tissue stroma tends to remain alive and replace the greater part of the dead muscle and fat cells (Peer 1955).

Despite general satisfaction with carefully cut muscle flaps, necrosis is a potential hazard, and has led to experimentation with other tissues which tend to survive free transplantation better. Although he was by no means the first to use a cartilage graft, A. L. Peer, in 1943, was the first to use diced autogenous cartilage grafts to repair skull defects (Peer 1943). Based on this work, Guilford, Wright and Draper (1958) employed a similar method to obliterate mastoid cavities. They used both human (homogenous) and bovine (heterogenous) cartilage. It would appear, in general, that autogenous cartilage is best as it tends to retain its cartilaginous structure whether or not it is transplanted with its perichondrium attached (Peer 1955). Merifield (1963) has confirmed this in cats, and shown, in addition, that any dead spaces become filled with dense connective tissue which unites the fragments

into a solid obliterative mass.

Autogenous bone grafts have been used in the same way. Tieffenberg, in 1948, was probably the first to use autogenous bone chips from the mastoid cortex and claimed good results with the method (Tieffenberg, 1949, 1964). However, Guilford mentioned, in 1958, that he was not satisfied with the method because too many of the chips remained inert and acted as foreign bodies (Guilford, Wright and Draper 1958). Schiller has more wisely employed autogenous cancellous bone and found this to provide an excellent "mastoid osteoplasty" (Schiller 1963). Compared with solid cortical bone chips, the cells in bone grafts of open structure largely survive, and the process of new bone formation serves to cement the graft firmly in place (Peer 1955).

Homografts and heterografts have been used in the case of both cartilage and bone, but in general, they have shown evidence of gross resorption. In an attempt to find a method of overcoming this problem, Guilford, Wright and Draper (1958), basing their work on Tucker's studies on cultured calf bone (1956), tried using living despeciated foetal calf bone paste which they were able to mould into the cavity. Measures such as these are still largely experimental, however, and are by no means in general use. Nevertheless, Härmä and Koskinen (1965) have reported good results with bovine bone, treated with ethylenediamine.

In spite of all these efforts, occasional grafts of both cartilage and bone have been partly reabsorbed, especially in children (Peer 1955) and in poorly vascular transplantation sites such as the mastoid cavity.

It is of interest, therefore, that Mahoney (1962), following up the work of Dodge and Craig (1953), and Spence (1954), has performed several tympanoacryloplasties by filling the mastoid cavity with methyl methacrylate. He found no sloughs and no tissue reaction to this material in 27 cases after nine months, and overcame the problem of the heat of polymerization by "irrigation with cool water until body temperature had been reached." However, one would consider it unlikely that the method will become popular, owing to a natural aversion to the unnecessary introduction of foreign materials into the body.

III SCIENTIFIC BACKGROUND.

1. The Normal Humerus of the Domestic Fowl.

The anatomy of the skeleton of Gallus domesticus has been briefly and rather incompletely described in the standard texts on the anatomy of the fowl (Kaupp 1918, Sisson and Grossman 1953, Bradley 1960, Marshall 1960), and very few accounts of its pneumatisation existed prior to the present century. John Hunter superficially mentioned it in 1774, and subsequently Jacquemin (1842), Sappey (1847), Selenka (1866), Campana (1875), Strasser (1877) and Wildermuth (1877) wrote on the subject. King (1957), in reviewing some of this early literature, mentioned that there was a remarkable diversity of opinion regarding which bones were aerated. From his own research, however, he concluded that the humerus is one of the most regularly and completely aerated of all the bones of the domestic fowl.

(i) General anatomy.

The fully developed humerus is stout and slightly curved, the distal end presenting two convex articular areas, the proximal end presenting a single ovoid articular area. On each side of the latter is a prominent tubercle, and on the medial side of the diaphysis, close to the medial tubercle and just below the proximal epiphyseal line, is an opening, the foramen pneumaticum (Opheim 1944).

On longitudinal section, the shaft is slender, has thin walls, and is slightly S-shaped, the circumference gradually increasing towards the extremities of the bone. The interior of the shaft presents a narrow

bony tube, the pneumatic tube (Bellairs and Jenkin 1960), which, leading from the foramen pneumaticum, opens into the intra-osseous, epithelium-lined pneumatic cavity of the bone. This cavity extends almost throughout the length of the adult shaft and is traversed, to varying degrees, by delicate bony trabeculae. The older the bone, the less the degree of trabeculation and the more the space resembles a large single air sac (Bremer 1940).

At each end of the shaft adjacent to the pneumatic cavity, one may see a persistent, narrow, dense, sponge-like network of endochondral bone with small marrow spaces between the trabeculae. In older humeri, however, both proximal and distal zones of thin cancellous bone become replaced by a few larger trabeculae, the air space then extending right out to a thin terminal plate of bone (Bremer 1940) which lines the deep surface of the articular cartilage. Diminution in width of the cancellous zones therefore represents the approach of completion of growth (Opheim 1944).

The humeri of younger fowls present similar, though more delicate, external features. However, in section, the appearance of the interior of the shaft varies with the stage of development. In the youngest chicks, no air space is seen, the shaft being occupied by bone marrow and presenting, in addition, the proximal and distal cancellous zones already mentioned. As development proceeds, the pneumatic cavity first appears as a small space just distal to the proximal zone of endochondral bone. The space then extends within the medullary cavity

over the entire transverse section of the bone, spreading proximally and distally, replacing the bone marrow. According to the age of the bird, the ratio of air space to bone marrow therefore varies, the former increasing, the latter decreasing with advancing development.

(ii) The Interclavicular air sac.

Air enters the pneumatic cavity of the humerus through the foramen pneumaticum having been conveyed there from the lungs via the interclavicular air sac. The pneumatic cavity of the humerus is, therefore, in reality, an extension of this sac.

The embryology of the chicken lung and air sacs has been thoroughly detailed by Locy and Larsell (1916). Air sacs appear in the chick during the first few days of incubation as terminal dilatations of the mesobronchi or secondary bronchi. The abdominal sac appears first, and the cervical, clavicular and thoracic sacs begin to grow out from the secondary bronchi on the 5th, 6th and 7th days respectively. The interclavicular air sac is originally formed from four parts, a mesial and a lateral moiety from each lung. Fusion of these parts occurs in the embryo by the 18th day and the septa thus formed break down between the 19th embryonic day and the 1st day after hatching to form the single structure of the adult.

The interclavicular sac in the adult is placed between and behind the two limbs of the furcula (the united clavicles), and is continued on each side as an axillary air sac (Bradley 1960). This extension passes out between the muscles of the axilla and communicates with the pneumatic

cavity of the humerus through the foramen pneumaticum. Attempts have been made to measure the maximum capacity of the clavicular air sac system of Gallus domesticus by using casts (King and Payne 1962). Difficulty was found in entirely filling all the ramifications, however, and although the main chamber of the clavicular sac filled well, the lateral chambers, including the interior of the humerus, filled less consistently. Nevertheless, it is thought that the total capacity is of the order of 90-100 mls.

(iii) Duration of the Pneumatisation process.

The time at which pneumatisation of the chick humerus commences is apparently subject to some variation. Selenka (1866) stated that the invasion occurs after the 22nd day. Wildermuth (1877), Blumstein-Judina (1905) and Eckert-Möbius (1938) stated that it does not occur until "several weeks" after hatching. Bremer (1940) described the first changes as occurring at the "porus pneumaticus" at a period ranging from 12-20 days after hatching. Opheim (1944) found that the youngest chicks in which the development of air spaces had begun were 36 days old, and the oldest in which no air space was found was 49 days old. Greven (1955) stated that pneumatisation begins immediately after hatching but Pratt and McCance (1960) reported that no extensive invasion of the marrow cavity is seen before 6 weeks in normal chicks.

The air spaces occupied about half the central marrow space between the 53rd-116th day in Opheim's series (Opheim 1944). However, Greven (1955) claimed that in his studies, this stage had been reached between

the 15th-35th day of life.

The time by which pneumatisation is complete also seems variable. The final stage of pneumatisation is said to coincide with the completion of ossification and growth (Opheim 1944). Latimer (1927) stated that there is a sex difference in the length of the chicken humerus, and that the growth of the female bone is complete by 110 days and the male bone by 140 days, while ossification is completed by about the 125th-172nd day of life. Eckert-Möbius (1938) said that pneumatisation was completed "in the course of some weeks", but in Opheim's series, the youngest chick with the air space filling the entire central marrow space, without loss of the cancellous zones, was 57 days old, while the youngest in which diminution of these zones had begun was 112 days old. However, in other chicks the cancellous zones were still present as late as 156 days (Opheim 1944). Pratt (1961) said that the cells of the growth cartilage of the chick femur are exhausted and have dissappeared by 155 days, and that the terminal plate of bone is formed by 190 days. The exact relationship of this to the chick humerus is uncertain, however.

The process of pneumatisation of the chick humerus has also been studied by Pratt and McCance (1960) under conditions of severe undernutrition. Cockerels were reared on an adequate diet for two weeks after hatching, and then given food sufficient only for survival. It was found that at the beginning of undernutrition no air cells had developed, but, three weeks later, pneumatisation was complete! It seems, therefore, that the whole process is greatly accelerated by

undernutrition.

(iv) Histological Features.

The long bones of birds present certain significant differences from those of mammals. In both groups, the first bone formed is of periosteal origin, but, in the chick, as soon as a midshaft periosteal shell has been laid down, the enclosed cartilage becomes excavated without being replaced by trabecular bone. Also, the arrangement of cartilage at the ends of the developing chick long bones is different, the pattern of its vascular tunneling is unlike that of mammals, and except in the tibiotarsus (proximal and distal ends), the tarsometatarsus and the carpometacarpus, sites which represent evolutionary fusion of bones which were earlier separated by synovial joints (Haines, Mohuiddin 1962), no separate epiphyseal centres of ossification develop. Further, there is a striking absence of endochondral ossification in all but the extremities of the diaphysis. One may also see viable cartilage cells far distant from their origin in epiphyseal cartilage. These points are sufficiently important to be dealt with in a little more detail.

In a 9 day chick embryo, the cartilaginous model of a long bone is arranged into a central diaphyseal cartilage and two peripheral epiphyseal cartilages. The central portion of the diaphyseal cartilage later becomes surrounded by a sleeve of perichondrial bone and undergoes hypertrophic changes. It is then excavated by the invasion of subperiosteal tissues so that a primary non-trabeculated medullary cavity is formed

while the shaft of periosteal bone is being laid down (Fell 1925, Pratt 1961). Subsequently, osteoclastic erosion of the inner surface of the diaphyseal wall results in a general expansion of this marrow cavity.

By the 57th day after hatching, this osteoclastic process becomes confined to localized sites and burrows into the shaft wall. It then extends circumferentially, so that sheets of bone come to lie within the medullary cavity. These are then subjected to remodelling and in this way intramedullary trabeculae are formed (Pratt 1961). Endosteal ossification takes place upon these trabeculae in the first instance, but later occurs throughout the entire medullary cavity, forming endosteal bone which is separated from the bone of periosteal origin by a cement line. The endosteal activity also serves to consolidate the trabeculae of endochondral bone and further cement lines mark this event.

Growth at the extremities of a chicken long bone is wholly cartilaginous and, on hatching, the epiphyseal region presents three cartilage zones (Wolbach and Hegsted 1952, Pratt 1961):-

1. a narrow, outer zone of articular cartilage composed of rather flatened cells with an eosinophilic intercellular matrix. This merges into the underlying zone of hyaline cartilage through a succession of cells of increasing size. There is no definite line of demarcation between the two zones.

2. a wide, basophilic zone of hyaline cartilage deep to the articular zone. This is clearly demarcated from the underlying growth

cartilage zone by virtue of differences in staining of the matrix of the two zones. The cells of the hyaline zone tend to become flattened adjacent to the growth cartilage, but are otherwise roughly spherical or oval, and arranged in groups.

3. an inner zone of growth cartilage. The proliferative zone of the growth cartilage of chicks is wide compared with that of mammals, the cells in it being markedly flattened. Although it has been stated that they are packed in columns (Wolbach and Hegsted 1952), this appearance is very vague indeed. The hypertrophic zone is certainly without any arrangement into columns. The cells become larger towards the diaphysis and are surrounded by an increased amount of matrix.

The vascular tunneling of the mature cells of the growth cartilage is widely and fairly uniformly spaced. Each vascular loop advances against a broad front of several cartilage cells, and columns of unabsorbed cartilage cells are seen between the separate loops. This pattern is quite different from the usual mammalian pattern where one sees the vascular penetration of orderly, adjacent columns of cartilage cells, but bears some resemblance to regions such as the distal end of the phalanx (man) where there are no secondary epiphyseal centres of ossification (Dodds 1930).

The cytological sequences which the cartilage cells undergo from the proliferative zone to the mature cell are basically similar to those in mammals, except that in the chick, degeneration is less complete preparatory to ossification, both in advance of and lateral to the

tunneling blood vessels (Wolbach and Hegsted 1952).

The cartilage columns between adjacent blood vessel loops become thinned by lateral "resorption", and an asymmetrical deposition of bone takes place on the walls of the vascular tunnels. One may sometimes see unabsorbed cartilage cells some distance from their origin from the growth cartilage. This is thought to arise because the deposition of bone along the walls of the tunnels and thereby around groups of cartilage cells delays their removal (Wolbach and Hegsted 1952).

As a result of these changes, a great complexity of arrangement is established which gives rise to the appearance of trabecular endochondral bone, the whole process being confined to the bone extremities. With maturity, the growth potential becomes exhausted and the growth cartilage disappears. The invading marrow tissues and advancing blood vessels then enter the hyaline zone of cartilage, and endochondral osteogenesis spreads through this zone. Finally, all that remains of the original cartilaginous end of the bone is the articular cartilage, lined on its deep surface by a terminal plate of bone (Pratt 1961).

It is upon this basic pattern that the pneumatisation process is superimposed. The axillary air sac enlarges and burrows through the loose areolar tissue between the axillary muscles, gradually approaching the upper end of the humerus. Small vessels pierce the perichondrium at several points around the circumference of the bone at this level and follow an oblique course through the bony cortex. The largest of these are on the medial side in relation to the axillary vessels, and groups of

them, mostly veins, persist in adult avian bones as accessory vessels to the main nutrient system (Doan 1922).

According to Bremer (1940), the connective tissue of the sac fuses with the periosteum and, at a variable period from 12-20 days, osteoblasts begin to disappear and increasing numbers of osteoclasts are seen in the area surrounding the exit of the proximal accessory vein. Trabeculae are gradually resorbed in this region, and the inner layer of periosteum becomes replaced by a delicate mesenchymal connective tissue. The changes progress inwards along the vessels and give rise to a track of mesenchymal tissue which passes obliquely through the bone cortex into the marrow cavity. The mesenchyme gradually becomes looser, and, according to Bremer, "irregular spaces or cysts" develop in it. Finally the air sac grows through this "loose, mesenchymal, cystic connective tissue" to enter the marrow cavity.

Similar changes are then seen within the marrow cavity. The process of retrogression of the marrow with its replacement by mesenchymal tissue extends progressively along the marrow trabeculae, reducing the marrow to isolated masses which later also disappear. The advancing air sac follows the mesenchyme, replacing it and branching out as it goes. Finally, as a result of decreased osteoblastic and increased osteoclastic activity, even the bone marrow trabeculae are progressively resorbed and the branches of the air sac fuse to form a more or less single air cavity which occupies the shaft. By this means, the bulk of the bone marrow is relegated to the two ends of the bone (Bremer 1940).

The air space slowly continues to grow at about the same rate as the bone until the final phase of development is reached. At this stage, the air space increases in size at the cost of the cancellous zones of endochondral bone so that the air space finally extends out to a thin, terminal shell of bone beneath the articular cartilages. Although Miller (1908) stated that when bones are fully pneumatic they are free from marrow and fat, this is probably not so. Persistent islands of fatty marrow can be found along the shaft and at the bone ends if searched for.

The whole process of development of the air space in the right and left humeri is symmetrical to a marked degree (Opheim 1944).

The lining of the pneumatic spaces is of great interest. The wall of the axillary air sac is composed largely of a simple cuboidal or flattened epithelium (Bremer 1940), and although Thomson (1923) stated that it was ciliated, this is probably so only near the openings to the lungs (Bellairs and Jenkin 1960). This rests on a delicate connective tissue with few blood vessels.

In like manner, the air space of the chick humerus is lined by a flat, single-layered epithelium resting on a zone of mesenchymal connective tissue which, in turn, rests on the bone. The thickness of this zone varies with the degree of pneumatisation and the situation at which it is examined. In front of the advancing air sac (pre-epithelial region), it is usually wide and irregular, merging into the bone marrow. Elsewhere, the expanding air sac tends to displace this tissue so that it

eventually remains as merely a thin layer beneath the epithelium. The inner layer of this tissue is said to be differentiated as the endosteum (Bremer 1940).

It was considered by Opheim (1944) that the epithelium of the pneumatic cavity of the humerus belongs genetically to the respiratory tract (via the axillary air sac). However, he stated that the underlying zone of mesenchymal connective tissue is not derived from the connective tissue of the wall of the axillary air sac, but from the bone marrow. He therefore considered this zone to belong genetically to the bone, and stated that the only element to enter the bone from without is the epithelial layer.

(v) Medullary new bone formation.

In 1934, Kyes and Potter (1934) reported for the first time that the marrow cavities of the tibia and femur of female pigeons become filled with endosteal bone during the laying cycle, and that this bone undergoes cyclic changes coincident with the maturation of the ovarian follicle. These osseous modifications could not be found in male pigeons.

This phenomenon has since been confirmed in pigeons (Bloom, Bloom, Mclean 1941; Riddle, Rauch, Smith 1944) and further studied in the sparrow (Pfeiffer, Kirschbaum, Gardner 1940), duck (Landauer, Pfeiffer, Gardner, Shaw 1941) and domestic fowl (Bloom, Domm, Nalbandov, Bloom 1958). In the case of laying chickens, there occurs an extensive deposition of trabecular endosteal bone which may be seen superimposed on the endosteal lamellar bone normally present. This bone is

irregularly and finely fibred, deeply basophilic, contains numerous irregularly-shaped lacunae (Pratt 1961) and, except for a narrow, persisting core of marrow, may almost fill the medullary cavity as a dense network of broad trabeculae. The changes are related to the calcium requirements for egg production and are governed by hormonal factors.

Although not normally seen in male birds nor in non-laying females, similar changes have been induced in both these groups by the administration of oestrogen in the cases of the pigeon (Pfeiffer, Gardner 1938; Bloom, Mclean, Bloom 1942), sparrow (Pfeiffer, Kirschbaum, Gardner 1940), and domestic fowl (Urist and Deutsch 1960). The degree to which these changes occur appears to depend primarily on the oestrogen level, although the influence of the androgens is the subject of some controversy.

Most reports concerning this unusual type of bone formation have been on the changes observed in the long bones of the leg. However, Taylor and Moore (1953) found that the phenomenon does in fact occur throughout the whole skeleton. In laying pullets, these authors were able to demonstrate that different bones are affected to different degrees and established the percentage which each bone contributes to the total skeletal medullary bone formation. The humerus was among those bones in which the phenomenon was present to a small degree only.

(vi) Blood Supply of Chick Long Bones.

In the past sixty years or so, the distribution of blood vessels in bones has received a great deal of attention, the basis of present

knowledge being laid by such workers as Langer (1876), Siraud (1895), Lexer (1903,1904), Lexer, Kuliga and Turk (1904), and Delkeskamp (1906). Since that time, numerous workers have studied the blood supply of various parts of the human skeleton in greater detail, while others have concentrated on common experimental animals such as the guinea pig (de Marneffe 1951), dog (Drinker, Drinker and Lund 1922; Johnson 1927; Rubaschewa and Priwes 1932), rat (Reichel 1947; de Marneffe 1951; McAuley 1958), and rabbit (Kistler 1934; Bragdon, Foster and Sosman 1949; de Marneffe 1951; Foster, Kelly and Watts 1951; Brookes 1957; Brookes and Harrisen 1957; Lemoine 1957; Morgan 1959; Trueta and Cavadias 1964).

However, apart from the limited work of Foote (1921) on the circulation within the bone substance of several members of the bird family, the only research carried out on the pattern of the intraosseous vessels of avian long bones is that of Doan (1922). Using an India ink injection technique he studied the radius and ulna of the pigeon to establish the details of circulation in the bone marrow. The humerus was purposely excluded from the investigation because of its pneumaticity.

No comprehensive work appears to have been carried out on the general intraosseous vascular pattern of the long bones of the domestic fowl.

The vascularization of cartilage, the function of cartilage vessels, and the mechanism by which cartilage canals are formed has been investigated by several workers (Eckert-Möbius 1924; Stump 1925; Haines 1933-4;

Hurrell 1934-5; Trueta 1957). However, apart from a mention of them by Lubosch (1924), Fell (1925) and Whisten (1940), no diagrammatic representation of the cartilage canals of the long bones of birds seems to have been published prior to that of Haines (1942). He claimed that they were essentially similar to those described for mammals and briefly described them as initially opening into the marrow cavities of the shaft. He felt that this continuity between the canals and the marrow of the shaft is lost in later stages, and that in later stages still, they dwindle away until, when the cartilage is reduced to a relatively narrow articular layer, no canals are left.

Welbach and Hegsted (1952) also felt that in the domestic fowl there is a communication between the canals of the hyaline zone of the cartilaginous epiphysis and the marrow of the shaft. They described the blood vessels of the nutrient system as invading the growth cartilage and extending through it to penetrate the hyaline zone where they branch and anastomose with each other. They were unable to decide whether the vessels entering from the shaft of the bone communicate with blood vessels from the perichondrium. They did, however, describe the terminal loops of the nutrient system. These were said to present 2-7 or more vessels of capillary dimensions when viewed in transverse sections. In addition they noted that the vessels in these sections are often elliptical and crescentic in shape, indicative of tortuosity and anastomoses.

(vii) Function of the Pneumatic Cells of Avian Bones.

If the function of air cells is dependent on their being in free

communication with the atmosphere, then any procedure which obstructs this communication must render them functionless, or at least seriously interfere with their function. The role which these air cells play in avian bones must therefore briefly be discussed.

As the air cells are a direct anatomical extension of the air sac system, the function of this system must be considered first. In 1908, Miller surveyed the major theories concerning the function of the air sacs of the pigeon (Miller 1908). He critically discussed suggestions that the air sacs act as resonatory organs for increasing the strength of the voice, that they act as manometric sense organs analagous to the swim bladder of fish, that they reduce the specific gravity of the bird, and that by selective filling of the air sacs, the centre of gravity is altered and equilibration during flight is facilitated. He also discussed theories that the air sacs may have a respiratory function, that they may serve as a store of air during flight, that they may offset the relatively large tracheal dead space by providing a "bellows" effect, and that they may be important in the regulation of body temperature by providing a surface from which evaporation of water can take place. In addition, Miller also mentioned ideas concerning superadded roles of specific groups of air sacs, such as erection of the feathers (subcutaneous sacs), increasing the freedom of movement of body organs (thoracic and abdominal sacs), mechanically assisting the muscles of the wing (axillary sacs), and assisting digestion (abdominal sacs).

None of these theories can be accepted without reservation and none

of them explains how the entry of air into the skeleton supplements or determines the function of the sac system. Certainly there is no doubt that respiratory pressure changes may be measured in the air cells within the humerus (Ojala 1957), but actual renewal of air within the bone must be extremely slow by virtue of the unyielding and blind nature of the space. Further, the blood vessels in the walls of both the air sacs and the air cells within the bones are derived from the systemic and not the pulmonary circulation.

In view of these problems, it is of interest to trace briefly the evolutionary history of the development of pneumaticity of the skeleton in relation to the acquisition of the power of flight in animals and the development of birds in general.

The presence of air spaces in bones does not appear to have been confined to birds. Some of the large dinosaurs, such as Brontosaurus and Atlantosaurus, had light, hollow bones which may have contained air cavities (Miller 1908). A tendency to develop pneumaticity was characteristic of the early archosaurs, and this was elaborated by their descendants the Pterosaurs (pterodactyls), which almost certainly had pneumatised bones (Marshall 1960). They took to the air on leathery wings and although some were gliders only, others acquired the ability to keep up sustained flapping (Carr 1964).

More important, however, was the development of the avian series. Archaeopteryx, a feathered, crow-sized animal dating from the Jurassic period 150 million years ago, possessed certain reptilian characteristics

but is generally recognised as the oldest known representative of the avian series. As such, it is interesting that none of its bones were pneumatised (de Beer 1954). The same applies (Miller 1908) to Hesperornis, a flightless bird of the Cretaceous period of some 120 million years ago which was perhaps five feet long (Wallace 1963), and had wings with a vestigial humerus only (Van Tyne and Berger 1959).

However, certain parts of the skull of Apteryx were affected by pneumatisation (Marshall 1960) and some degree of pneumatisation was also seen in the bones of the new extinct New Zealand Moas (Miller 1908).

Among recent birds, the degree of pneumaticity of the skeleton varies considerably. In general, the large, strong-flying birds have greatly aerated bones, but so also do some poor fliers, such as the domestic fowl and the hornbill, and many of the flightless birds, such as the Ostrich, Emu, Cassowary, Kiwi and Rhea. In contrast, many small birds, which are excellent fliers, have marrow-containing bones, as do many diving birds and birds with aquatic tendencies (Headley 1893). The relationship of bone pneumaticity to the power of flight per se is, therefore, not a simple one.

The degree of aeration of the skeleton seems to be more closely connected with the length of the bones in relation to the size and weight of the bird. Headley (1893) concluded that as the wing lengthened phylogenetically, the bones became larger in girth as well as in length. This would have resulted in a considerable weight increase had not the bones become air-filled. In preventing a weight increase, the

pneumatisation process helped to avoid the development of an additional large work load for the flight muscle system, yet at the same time enabled the strength and form of the bones to be maintained. As the importance of these factors is minimal in the case of small bones, it is not surprising that the skeleton of small birds, and the small bones of some of the larger birds, are not pneumatic.

2. The Pneumatic System of the Human Temporal Bone.

The detailed features of the gross and microscopic anatomy of the human temporal bone have been studied by many workers. Several aspects of their work require consideration in order to establish that the pneumatic system of the humerus of Gallus domesticus resembles that of the human temporal bone sufficiently both in structure and development, for there to be, in fact, good grounds for applying observations from the experiments on the humerus of the domestic fowl to conditions in man.

(i) Anatomy of ventilatory pathways.

No attempt will be made to describe the gross anatomy of the air cell system of the temporal bone in detail, but mention must be made of the outlets of the various air cell groups so that an idea of the pathways of ventilation of the system may be obtained. It is, of course, realised that the pneumatic conditions in the mastoid vary from individual to individual, but in general, the primary pathways of ventilation have been established by East and Forester (1939):-

(a) the mastoid and antral cells, being outgrowths of the antrum,

are ventilated from it.

(b) the Petrous air cells, other than the antral cells already mentioned, open either into the middle ear or the Eustachian tube in the following manner:-

1. The subtubal cells usually open into the middle ear below the Eustachian tube, but may also open directly into the tube.

2. the postcarotid cells generally open into the middle ear in association with the subtubal cells, but may rarely open into the Eustachian tube.

3. the precarotid cells may open into one of three areas - the mouth of the Eustachian tube, the middle ear between the Eustachian tube and carotid canal, or the subtubal cells.

4. the precochlear cells open into the middle ear at a situation between the upper part of the mouth of the Eustachian tube and the tip of the cochlea. The supracarotid cells, when present, are usually an extension of this group (East and Anson 1949).

5. the supracochlear cells open into the middle ear a little medial to and above the processus cochleariformis, and anteriorly to and a little below the geniculate ganglion.

Secondary ventilatory pathways are provided by a complex inter-communication of groups of petrous cells with each other. The post-carotid cells may communicate with both the subtubal and precochlear cells, and the supracochlear group may be continuous with the latter. The supracarotid cells, when present, are an extension of the precochlear

group, and it is thought that the supracochlear cells may occasionally communicate with the antral cells through and over the arch of the superior semicircular canal (East and Anson 1949).

Another interesting, and potentially important, communication sometimes occurs between the mastoid and hypotympanic cells. Almour (1933) found evidence of this in 100% of 24 well pneumatised bones, but East and Forester (1939) found it in only a few cases. Lindsay (1940) found such a tract extending from the hypotympanum to the mastoid in 13% of dissected bones, and Singleton (1944) found it in 9%. It appears that the tract most commonly passes from the hypotympanum medial to the facial canal and ends in the retrofacial, infralabyrinthine, or mastoid tip cells. Himalstein (1959) claimed that this tract is not an anomaly or mutation, is probably more common than generally thought, and has a precedent in phylogeny.

It can, therefore, be appreciated that the petrous cell system is ventilated via several openings from the middle ear and Eustachian tube, and that considerable communication exists between cell groups. This indicates that obliteration of an isolated group of petrous cells would not block the ventilation of other areas, and shows that a total middle ear and protympanic obliteration would be necessary to isolate the whole petrous system. However, although the antral cells may communicate around the semicircular canals with cells deeper in the petrous bone (Rainer 1938), the mastoid cells provide a different situation. This group is extensively ventilated only via the antrum, so that obliteration

of the antrum per se would be expected to cut off the entire mastoid portion of the air cell system, except in those cases where a Hypotympano-mastoid ventilatory pathway is present and supplies a large number of cells. A more extensive obliteration which included the retrofacial area would certainly be effective in all cases however.

Thus, the fact that areas of mastoid and petrous air cells may be completely cut off by blockage of one or more of the normal ventilatory pathways, and the fact that the whole pneumatic system of the humerus of the domestic fowl can be isolated by blockage of the foramen pneumaticum, means that on an anatomical basis there are good grounds for applying observations from the experiments on the humerus of Gallus domesticus to conditions in the human temporal bone.

(ii) Histological features.

According to Bast and Forester (1939), most air cells are normally formed in young growing bone before bone marrow is formed. They felt that only the antral, postcarotid, and part of the supracochlear air cells, invade bone which contains bone marrow. However, Eggston and Wolff (1947) stated that during the period of development of the mastoid process, the bone is filled with marrow in all areas except the region of the antrum and developing parantral cells. Ham and Leeson (1961) did not agree with either of these views. They claimed that the mastoid process and the bone surrounding the inner ear is normally quite filled with haemopoietic marrow in early life, and that this marrow is invaded by air sacs continuous with the antrum, middle ear cavity, and Eustachian

tube, during the process of pneumatisation. They made no exception to this rule for any area of cells.

The process of pneumatisation is histologically the same in man as in the humerus of the domestic fowl. Air cells do not invade bone or bone marrow directly. The primitive marrow first disappears and is replaced by a mesenchymal type of tissue which gradually beomes loose, and is finally replaced by the ingrowing air sac. The surrounding bony trabeculae then resorb to permit epithelial expansion and enlargement of the pneumatic cells (East and Anson 1949), while the marrow space recedes towards the mastoid tip and petrous apex regions. However, islands of fatty marrow may persist scattered through an apparently completely pneumatic adult mastoid (Eggston and Wolff 1947).

The lining of the pneumatic spaces which communicate with the tympanic cavity of the human temporal bone also must be compared with that of the humerus of the domestic fowl. Politzer (1883) described the walls of the spaces as being covered by a delicate nonciliated membrane closely united to the periosteum and in continuity with the lining of the tympanic cavity. Kraing (1924) considered that the lining is composed of an endosteum intimately adjoined by epithelium, and thought that in the adult there is no characteristic tunica propria containing blood vessels, nerves and glands. He therefore preferred not to use the term "mucous membrane" for the lining of the pneumatic cells. Ruedi (1937) also distinguished a periosteum (endosteum) which he felt was differentiated from mesenchyme, but described this as being separated

from a flattened nonciliated epithelium by a layer of foetal myxomatous tissue. He considered that the subepithelial portion of this layer differentiates into a tunica propria and used the term "mucous membrane" for the combination of epithelium and tunica propria. Ruedi felt that the submucosal layer of the myxomatous tissue disappears as pneumatisation proceeds so that the tunica propria comes to adjoin the periosteum Ojala (1950) considered, however, that the tunica propria also becomes thin and atrophic beneath the epithelium, so that the entire layer of foetal myxomatous tissue eventually becomes greatly reduced.

Although the relevant histological features of the air cells of the human temporal bone have been considered only briefly, it is apparent that there is a close resemblance between these features and the histological structure of the air cells of the humerus of Gallus domesticus. It is therefore considered that on a histological basis there are good grounds for applying observations from the experiments on the humerus of the domestic fowl to conditions in the human temporal bone.

(iii) Duration of the Pneumatisation Process.

A brief survey of the literature reveals some disagreement concerning the actual time at which mastoid pneumatisation commences. Hamar (1902) found "air cells" to be present at the end of foetal life, Rouviere (1910) found them in an eight month foetus, and Mouret (1913) reported their presence as early as the seventh foetal month. McMurrich (1923) and Gray (1930) stated that they appeared at or slightly before birth, and Bast and Anson (1949) found the earliest signs of developing mastoid

air cells in a 135mm foetus (34+ weeks). However, other workers have felt that although the antrum is present by the end of foetal life, mastoid air cells do not begin developing until birth or soon afterwards. For instance, Wittmaack (1918) stated that no air cells exist before birth, and Barth (1930), basing his conclusions on the examination of serial sections of temporal bones from the second foetal month to seventeen years, found that mastoid air cells developed "soon after birth". Nishimura (1936) more or less agreed with this and concluded that mastoid pneumatisation begins during the first months after birth. However, Eggston and Wolff (1947) felt that mastoid air cells do not develop until about the third year of life and Quain (1915) went so far as to state that they were not formed "till near puberty".

Unlike the case of the humerus of Gallus domesticus, it has proved impossible to assess the time at which pneumatisation of the temporal bone is half completed because of the anatomical arrangement of the region, but attempts have been made to fix the average time by which the process is complete. Although Wittmaack (1918) felt that the major part of air cell development takes place during the first five years of life, he considered it probable that it also goes on very slowly for the whole of life. However, Nishimura (1936) was of the opinion that the pneumatisation process reaches the mastoid apex during the third year of life and it almost complete by the 7th-8th year, and Eggston and Wolff (1947) considered it continues only until puberty.

Unfortunately there have not been any studies concerned with the

date of the beginning or termination of pneumatisation of the petrous pyramid, nor with the influence of sex or malnutrition on the duration of air cell development in general. One may only conclude that the mastoid air cells develop from the antrum at or soon after birth and that the process is virtually completed by the 5th-10th year of life (Diamant 1940). An analogy can therefore reasonably be drawn between the fully developed humerus of Gallus domesticus and the temporal bone of man after puberty, and between the partially pneumatised chick humerus and the temporal bone of a child of up to five years.

3. Mechanism of Pneumatisation.

The mechanism of pneumatisation of the human temporal bone has been extensively studied by means of gross, radiological, corrosion and histological methods, using both normal and diseased bones of ages varying from embryonic to adult life. Nevertheless, the subject remains somewhat confused and theoretical. It is for this reason that the more controlled research using the chick humerus is welcome.

The theories arising out of all this work may be divided into three broad groups, but as the literature on the subject is now so vast, only the most significant work will be reviewed.

(i) The pneumatising capacity of the mucosa.

In 1877, Wildermuth claimed that the fibrous tissue layer of the chicken air sac combines with the periosteum to form processes which eat into the bone cortex "like the penetration of a pathological neoplasm"

(Wildermuth 1877). Wittmaack (1918) developed this theory in relation to the temporal bone by postulating that the mucous membrane of the middle ear has a "pneumatising capacity" per se. He defined the "mucous membrane" as consisting of the epithelium and the entire underlying connective tissue and claimed that by the development of hypertrophic and hyperaemic changes in the subepithelial connective tissue, osteoclastic activity is increased and the bony walls enclosing the marrow spaces become absorbed. He stated that the subepithelial tissue then pushes into the opened marrow spaces displacing the marrow, and felt that the final stage involves the atrophy of this tissue and the invasion of the preformed cavities by the epithelium.

However, Meyer (1931-a), and Bast and Forester (1939), considered that only the epithelium is active, and felt that the subepithelial tissue cannot be held responsible for the preformation of the bony spaces. Following a series of experiments on the development of the air space in the chick humerus, Opheim (1944) supported this by concluding that the epithelium is the only tissue to enter the bone from without. He believed that re-organisation and retrogressive changes occurring within the bone during pneumatisation depend on the interaction of chemical-hormonal influences between the epithelium and the marrow.

(ii) The role of growth and moulding of the bone itself.

On the basis of histological work, Rüedi (1937) considered that pneumatisation is primarily a mesenchymal process involving an initial

phase of periosteal (endosteal) activity leading to the formation of bony spaces filled with a loose connective tissue, and a later phase of physiological atrophy of this filling tissue leading to air space formation. He considered that pneumatisation is intimately related to changes occurring during the growth and moulding of the bone itself, and that the epithelium does not determine the formation of the preformed spaces but serves only to line them when they have been formed.

Eckert-Möbius (1938) held a somewhat similar view regarding the importance of bone growth but felt that the formation of bony spaces depends on the relationship between stress, disuse, and bone nutrition. He postulated that the inner and outer cortex of the mastoid are subject to functional stress and therefore have a good blood supply, but considered that the diploe, being less subject to stress, atrophies until a nutritional equilibrium, determined by function, is reached.

In 1940, Bremer, working with the chick humerus, attributed the bony changes to hormonal factors. He considered that oestrogens taken into the chick, by retraction of the yolk sac before hatching, act on the parathyroid glands to produce a temporary, localised condition similar to osteitis fibrosa cystica at the upper end of the humerus, thereby affording a mesenchymal pathway for the air sac to enter the bone (Bremer 1940).

Schwarzbart (1959) has revived and extended the mesenchymal concept of pneumatisation on the basis that there is a spectacular histological discrepancy between the ciliated columnar epithelium of the Eustachian

tube medial to the isthmus, and the flattened epithelium of the tympanic cavity (Schwarzbart 1958). He felt that there is little evidence that the epithelium of the Eustachian tube grows into the air spaces of the temporal bone, and observed that the narrow spaces first transform into cavities filled with a "gelatinous embryonal tissue" which then becomes almost acellular centrally, and flattened and endothelium-like adjacent to the bony walls. Upon the entry of air into the upper respiratory tract at birth, the acellular, almost amorphous tissue shrinks and is replaced by air, while the flattened, mesenchymal, endothelium-like cells become the lining of the air spaces. He considered the "foetal gelatinous connective tissue" to be responsible for "preparing and up-building the process of pneumatisation" (Schwarzbart 1960). Buch and Jorgensen (1964) disagreed with Schwarzbart's idea that the isthmus of the Eustachian tube is the definitive boundary. After finding areas of ciliated stratified columnar epithelium in the middle ear, they considered that the tympanic cavity and not the isthmus of the Eustachian tube should be considered as the transitional zone between the endodermal and the mesodermal germinal areas.

(iii) The influence of mechanical factors: muscle traction and atmospheric pressure.

In 1877, Strasser, speculating on the cause of pneumatisation of the chick humerus, maintained that the bony cortex was weakened at a point between the shoulder joint and the attachment of the muscles to

the upper end of the humerus because this region was subjected to movement during osteogenesis. He considered it possible that the weakness of this area facilitates the entry of the air sac into the bone (Strasser 1877). Proetz (1922) applied the idea somewhat differently to the mastoid process and stated that muscle pull opens the outer cortex and facilitates air cell formation. Loebell (1937) found support for this from a study of torticollis material and claimed that muscle traction promotes both pneumatisation and longitudinal growth of the mastoid.

In addition to his thoughts concerning the role of muscle traction, Strasser (1877) considered that the air sac enters the chick humerus by simple pressure. Krainz (1924) apparently agreed with this idea in relation to the temporal bone. He stated that atmospheric pressure actually pushes the epithelial sac into the marrow spaces, and also postulated that the bone marrow disappears under the same influence. He felt that the pressure of the air causes venous stasis in the subepithelial connective tissue, and that this gives rise to the development of oedema which extends to adjoining marrow spaces. The oedema in these spaces results in further pressure which is said to lead to a pressure atrophy of free marrow cells.

However, Opheim (1944) considered it improbable that the epithelium or the development of the air cells is forced forward by atmospheric pressure and based this view on his observations that the foramen pneumaticum of the chick humerus appears before hatching, that is, before

atmospheric pressure can play a part. He felt that although atmospheric pressure is a mechanical prerequisite for pneumatisation because the air space can only increase in size if no negative pressure arises, it is not the cause of the process.

Experiments concerned with obstructing the foramen pneumaticum of the chick humerus have not really clarified this point. Opheim (1944) destroyed the foramen by galvanocautery in chicks in which development of the air spaces "had not yet presumably begun", and Greven (1955) used paraffin and dental cement to achieve the same effect. This certainly prevented air from entering the bone by providing a mechanical blockage of scar tissue, paraffin, or dental cement, but it also destroyed the epithelium and led to an inflammatory reaction in the area. The theoretical question regarding the effect of blocked ventilation on pneumatisation, therefore, remained unsolved.

4. Pathological Reactions in Air Cells.

A great volume of literature now exists concerning the effects of inflammation and tubal occlusion on the pneumatic system of human and various animal temporal bones. It is considered necessary to review this work to gain an insight into the changes which might be expected to occur in the air cells after partial exenterative and obliterative surgical procedures such as are the subject of this investigation.

(i) Effects of Inflammation.

(a) Inflammatory reactions in the fully developed pneumatic system.

It was originally believed that the compact mastoid bone was a product of chronic or recurrent inflammation. Schwartze and Eysell (1873), Haymann (1912), Stewart (1928), Husik (1932), Opheim (1941), Ojala (1953), Tamari and Sganto (1954), Friedmann (1955, 1957, 1963), Tumarkin (1957, 1959), Weller (1958), and Senturia et al (1962) are among the many workers in this field who have done much to clarify this problem.

The initial effects of the inflammatory process on the epithelium and its underlying connective tissue are hyperaemia, oedema, thickening and cellular infiltration, and these may result in obstruction of the openings of groups of air cells. As more damage occurs, the lining epithelium may be destroyed. However in 1955, Friedmann reported another interesting and important finding. The lining of the guinea pig bulla, when not destroyed by the inflammatory reaction, may be converted into a tall columnar, ciliated mucosa complete with goblet cells and gland-like structures showing clearly-staining mucous secretion (Friedmann 1955-b). Senturia et al (1962) confirmed this in dogs by discovering that late in the course of the inflammatory reaction resulting from electrocautery of the nasopharyngeal orifice of the Eustachian tube, simple glands might be found buried in the thickened fibrotic lamina propria of the bulla. Friedmann (1963) has since demonstrated this secretory transformation in bone chips removed at operation from the human mastoid air cell system as well as in specimens of middle ear mucosa. It is still uncertain, however, whether this represents a

metaplastic transformation or an extension of the columnar epithelium of the tympanic orifice of the Eustachian tube into the pneumatic system.

The islands of bone marrow within the mastoid are also affected. The reticulum swells up, blood forming elements disappear, fat cells atrophy and degenerate, and the resultant mass of tissue is later replaced by granulation tissue. This results in an eventual fibrosis of the marrow, a process which Stewart (1928) claimed could be reversed.

In addition, changes occur within the lumina of the air cells. They first fill with a serous transudate, but, as the inflammatory reaction progresses, a purulent cellular exudate comes to occupy the spaces. If this does not drain away, it becomes stagnant, less fluid, and "fibrinised" (Stewart 1928). Ojala (1953) was of the opinion that only those cases with evidence of severe inflammation develop an exudate containing fibrin, and he also thought that it is only this type of exudate which undergoes organisation. The most common areas of the cell system to be involved are the apical cells and the small cells around the antrum. It is here that one may first see air spaces partially filled with masses of granulation tissue lined by remnants of epithelium, presenting an over-all picture of small epithelium-lined cavities separated by columns of connective tissue (Ojala 1953). Repetition of the whole process leads to progressive reduction of the lumen and eventual fibrous obliteration, but it is said that before this can occur, the epithelium must have been destroyed.

As a result of the inflammatory changes, the physiological

equilibrium between bone apposition and bone resorption is disturbed. Resorption dominates when the inflammatory reaction is intense, and bone is removed as a result of osteoclastic activity accompanied by the ingrowth of blood vessels and halisteresis (Stewart 1928). Later, however, new bone formation dominates, being greatest in the fibrosed marrow spaces and in the cells which are wholly or largely filled with granulation tissue. The new bone is laid down by osteoblastic activity both at the mastoid cell wall and within the fibrous tissue filling the air spaces (Tamari and Ssanto 1954), and may present a reticular or a lamellar pattern. Stewart (1928) thought that the former represents a response to a great stimulus, and that the latter, being formed less quickly, represents the response to a chronic low grade stimulus. Friedmann (1957) more or less agreed with this and described an active, luxuriant new bone formation in acute or subacute mastoiditis, and the alternate absorption and deposition of bone in cases of chronic otitis media, leading to the formation of a mosaic pattern with irregular cement lines.

Although apparent new bone formation has been observed by many workers in the case of the human mastoid air cell system, the fact that it may occur does not seem to have been universally accepted. Evidence from controlled animal experimentation is therefore interesting. In 1912, Haymann inoculated the bulla of the guinea pig with virulent organisms and, among other findings, described circumscribed or diffuse areas of ossification (Haymann 1912). In 1931, Meyer, using both

chemical agents and bacteria, experimentally produced an inflammatory process in the middle ear spaces of apes and observed new bone formation in one case of chronic or recurrent inflammation (Meyer 1931-b). Four years later, Wirth showed that processes of bone formation could be seen on the wall of the bulla and on the promontory as a result of experimental middle ear inflammation in rabbits (Wirth 1935). Friedmann (1955-a) also noted new bone formation in the bullae of guinea pigs whose ears had been infected with a suspension of pseudomonas pyocyanea. In the chronic phase of the infection, the reaction led to thickening of the bullae and sometimes to bony obliteration. It was evident, however, that different organisms gave rise to different degrees of this phenomenon. Senturia et al (1962) found evidence of new bone formation in the bullae of dogs as a result of the inflammatory reaction following electrocautery of the nasopharyngeal opening of the Eustachian tube. He noted this in about 50% of his series after 14 days.

This work indicates that it is quite possible for the pneumatic system to become filled and obliterated by fibrous tissue, or to decrease in extent as a result of new bone formation in cases of chronic or recurring inflammation. Further, as the inflammatory process seldom extends uniformly throughout the system, the sclerotic changes tend to be typically patchy in distribution, and a varying number of cystic cavities remain (Ojala 1950). Repneumatisation can only occur if the inflammatory reaction has subsided, if tissue proliferation has ceased, and if aeration through natural channels is still possible. It is felt

that if cells are cut off by cicatricial tissue or new bone formation this cannot occur.

(b) Inflammatory reactions in the developing pneumatic system.

Hartmann (1879), Bezold (1893), and Heine (1904) were among the first to express the view that a sclerotic mastoid could exist without any evidence of a previous inflammatory process, and in 1910, Cheatle postulated that the anatomical conditions seen in the "infantile" type of temporal bone were factors in producing chronic suppuration and not the result of it (Cheatle 1910-a and b). There soon arose a host of theories concerning the factors responsible for the primary arrest of air cell development. Inevitably, these were based on already existing theories of pneumatisation which had little factual basis.

Wittmaack (1918) believed that the pneumatising capacity of the mucous membrane might be affected by pathological changes resulting from an uninfected foreign body (such as meconium or vernix caseosa) entering the middle ear via the Eustachian tube prior to the completion of the pneumatisation process. He also felt that the mucosa might similarly be damaged by suppurative otitis of infancy and thereby fail to pneumatise the bone normally (Almour 1933-a). On the other hand, Eckert-Mobius (1938) explained the arrest of pneumatisation on the theory that an inflammatory process causes a stimulus which prevents the bony atrophy which he felt was the primary factor in permitting air cells to develop. However, Krainz (1924) considered that arrested pneumatisation was due to the formation of scar tissue or new bone formation

as a result of an inflammatory reaction. He postulated that this acted as an unyielding obstacle to the pneumatisation process.

In effect, all these workers ascribed the arrest of air cell development to an inflammatory process occurring in the developmental period. Nevertheless, one cannot say that this theory has been proven, and in fact many workers have claimed that primary sclerosis is determined by genetic and not purely pathological factors (Albrecht 1924, Schwarz, M. 1929). Experiments to examine the inflammation theory were therefore devised in 1944 by Opheim. Using the chick humerus, he investigated the effects of a sterile foreign body (paraffin) placed in the developing air space and in the as yet un-pneumatised bone marrow. It was found that air space formation was not arrested except in so far as the paraffin occupied a given area of the available space. The conclusion was drawn that it is highly improbable that a slight foreign body reaction can totally arrest the development of the air spaces (Opheim 1944). Ojala (1957) attempted to inject bacteria into the developing chick humerus to simulate the conditions of an infantile otitis but was unable to obtain any results. The chicks either died within a few days or, if the dose was reduced, no inflammatory reaction resulted.

Despite the exact mechanism involved, it is found that in cases of primary arrest of pneumatisation, the changes are of a generalised rather than a patchy sclerosis, the overall architecture being characteristically small (Cheatle 1910, Herrmann and Riehm 1961), and the distance

between the anterior boundary of the sigmoid sinus and the osseous posterior meatal wall being reduced. This is, of course, in contradistinction to the anatomical conditions in cases of secondary sclerosis.

(ii) Effects of ventilatory obstruction.

(a) Effects of ventilatory obstruction on the fully developed pneumatic system.

In 1869, Politzer set forth his theory that Eustachian obstruction results in the absorption of oxygen from the enclosed pneumatic system because of a difference between the gas pressure in the air space and the middle ear tissues. He felt that this lowers the total pressure in the system and that the negative pressure so produced leads to hyperaemia and favours the exudation of fluid from the vessels into the tympanic cavity by a process of sterile transudation (Politzer 1883). Hyperaemia, impaired mobility and retraction of the tympanic membrane in the early stages, and the appearance of air bubbles rising in the fluid after paracentesis were taken as clinical support for the concept.

Several workers were not convinced. Blegvad (1941) considered that the transudation ex vacuo theory was wrong because he doubted that hermetic sealing of the tube actually occurred in man except in some cases of neoplasm and severe scarring. He also considered that experimental attempts at producing the occlusion inevitably gave rise to an inflammatory reaction which extended to the tympanic cavity, and thought that the effusion was in fact an inflammatory exudate. He was not alone in this view. Van Dishoeck (1941) stated that Neumann, Brieger, Görke,

Kümmel, Manasse and others were also of the opinion that the fluid did not represent a transudation ex vacuo but was an inflammatory exudate.

Experimental support for Politzer's theory was difficult to obtain because of the lack of a satisfactory method of closure of the Eustachian tube. For instance, Laurowitsch (1913) tried closing the tube with bone or horn, Beck (1914) initially used wooden blocks but later tried electrocautery (1919), and Claus (1930) tried injecting paraffin into the lateral wall of the tube in addition to cutting the tensor palati. All these methods failed because an inflammatory reaction invariably developed. In 1934, G. Holmgren had more success. He surgically ligated and thereby closed the pharyngeal end of the Eustachian tube in dogs and was able to place a manometric device in the bulla and record a progressive reduction in pressure (Holmgren 1934). However, the manometer was sensitive to temperature changes and this gave rise to severe criticism of his results. In 1940, L. Holmgren repeated the experiment with an improved method and found that in cases where a hermetic seal was obtained, he was able to confirm the development of a negative pressure and obtain an effusion free of any evidence of infection. It is important to note, however, that a period of occlusion of more than 6 days was necessary before these changes developed (Holmgren 1940).

Further support for the theory was obtained by gas analyses of the air in the closed tympanic cavity. Matsumura (1955) aspirated air through the tympanic membrane and, by using a modified Krogh's method, was able to show that the oxygen vol.% is normally 14-17%, but that in

cases of Eustachian tube stenosis it varies between 9-15%. This was taken as confirmation that oxygen is absorbed when the pneumatic system becomes closed.

Along entirely different lines, Van Dishoeck (1941) was also able to show that a negative pressure develops after blockage of the Eustachian tube. Utilising the evidence obtained by Thompson, Howe and Hughson (1934) that an increase or a decrease in middle ear pressure impairs the conduction mechanism of the cat's ear, he developed the Pneumophone and was subsequently able to demonstrate that clinical obstruction of the tube results in a negative pressure of -50cm. of H_2O, and a hearing loss of 20 db. for tones under 4000 cps. Rahm, Strother, Lucchino and Gulick (1958) confirmed experimentally in cats that this loss of auditory sensitivity is mainly due to distortion of the tympanic membrane as a result of the negative pressure in the air system.

In 1883, Politzer admitted that "rarefaction of air in the middle ear caused by closure of the tube does not always produce a transudation of serous fluid into the tympanic cavity". It is also of interest that although Van Dishoeck (1941) often found a maximum negative pressure of -50cm. of H_2O following Eustachian obstruction, he did not always find fluid. On the assumption that the total volume of the pneumatic system was only about 2 ccs. and that a reduction of only 1/20th. of this volume was necessary to decrease the pressure to -50cm. of H_2O, he suggested that swelling of the mucous membrane per se, blocking of groups of air cells by the mucosal swelling, and accumulation of fluid, should

easily make up for any decrease in volume due to a loss of oxygen by absorption. He therefore thought that the development of a negative pressure could not be ascribed to oxygen resorption alone. However, it would appear that his assumption that "the total volume of the pneumatic system is about 2ccs." is invalid. On the basis of direct volume determinations on sectioned temporal bones using a special vacuum technique to fill the air spaces with water, Silberger (1950) found that the volume of the air cell system varies from 6-35ml. Flisberg, Ingelstedt, and Ortegren (1963-a) made volumetric determinations using a method based on the general gas law and obtained figures which varied from 0-25ml. They postulated that a negative pressure develops more rapidly in a small air cell system than in a large one and suggested that the role which the tympanic membrane plays as a pressure regulator of the middle ear is greater in a small system (1963-b).

A more recent theory regarding the cause of the decrease in the oxygen concentration and the lowering of pressure follows from the observation that distension of the tympanic membrane may be noted during inhalational anaesthesia commenced after passage of a cuffed endotracheal tube under local anaesthesia, so that the passage of anaesthetic gas up the Eustachian tube is prevented (McGuckin 1964). Taking this knowledge into account, and finding that, with an apparent blockage of the Eustachian tube, the oxygen concentration of the enclosed air in simple mastoid cavities was the same as that of room air, Adams (1953) postulated that the development of a negative pressure may be the result of

an inhibition of normal gas secretion in the middle ear cleft.

Reports of histological changes which result from simple ventilatory blockage of the fully developed pneumatic system under atmospheric conditions have usually been complicated by the finding of associated inflammatory changes. Recent experimental attempts to obtain further information have employed galvanocautery to occlude the tube. Senturia et al (1962) failed to achieve closure with this method, but Sala and De'Stefani (1963) were successful. Nevertheless, all these workers have reported a considerable inflammatory reaction as occurring in the middle ear because of the operative interference.

Because of these problems, the histological changes which result from the more marked degrees of negative pressure found in cases of otitic barotrauma and reproduced experimentally in animals using pressure chamber techniques merit consideration. The function of the Eustachian tube is of major importance in these circumstances. Armstrong and Heim (1937) found that whereas ventilation of the middle ear can be entirely passive during ascent (decompression), the tube must be opened by active muscular action in descent (recompression) if pressures are to be equalised because of a "flutter-valve" mechanism of the Eustachian tube. In man, opening of the tube becomes progressively difficult as the middle ear pressure is decreased until at a relative negative middle ear pressure of 80-90mm. Hg. it becomes impossible. Flisberg, Ingelstedt and Ortegren (1963-c) have also studied this mechanism and have stated that "locking" of the tube can be provoked by a middle ear pressure of

-30mm. Hg. in some subjects, whereas in others the phenomenon does not take place until -50mm. Hg. or below. In any event, because of this mechanism it is not surprising that most cases of aerotitis media occur during the phase of recompression (Hyde 1952).

The histopathological changes resulting from such conditions in man have been reported by several workers. Armstrong and Heim (1937) found evidence of passive hyperaemia of the mucous membrane progressing to the features of a traumatic inflammation with a serous effusion in severe degrees of acute otitic barotrauma. In chronic cases, they found that the mucosa becomes chronically congested and thickened. Fowler Jr. (1945) described somewhat similar changes. In acute cases, he noted oedema of the mucosa, congestion of the vessels, submucosal extravasations of blood, and bleeding into the air spaces. The fluid produced was either serous and clear, or bloody and mucoid. In recurrent cases, however, he reported the finding of loose granulation tissue in the cavities of the middle ear and mastoid, and in chronic cases there was an excess of fibrous tissue. Whether these changes were due to infection or to negative pressure per se is uncertain.

In animals, the reported changes are similar. Dickson, McGibbon and Campbell (1943) found congestion, haemorrhage, and cellular infiltration of the mucoperiosteal lining of the bulla of the cat, while Chang, Margaria and Gelfan (1950) reported bleeding into the tympanic cavity, antrum and mastoid cells of monkeys as a result of negative pressure.

Apart from reports concerning the sterility and varied appearance of the fluid released in otitic barotrauma, little information has been available about its chemical nature until recently. In 1961, Senturia et al studied this problem and found very few blood cells in the fluid, no mucous, and no glycoprotein. He also showed by electrophoretic studies, that all the common fractions of blood serum were present in approximately the same proportions as in serum, and concluded that the effusions have blood serum as their source (Senturia et al 1961).

The general opinion has been that negative pressure per se is the cause of these changes. It appears that both the degree of the negative pressure and the duration of its effect are important. In confirmation of this, Flisberg, Ingelstedt and Ortegren (1963-b), using a mastoid puncture cannula, found that the transudation of a clear yellow fluid could be produced in 5 minutes at a pressure of -100mm. Hg., but that at a pressure of -20 to -30mm. Hg. it took 15 minutes. Chang, Margaria and Gelfan (1950) found that a low negative pressure which does not ordinarily cause haemorrhage may do so if allowed to persist for a longer time.

However, in 1945, Fowler Jr. raised the question whether anoxia at height may be the cause of the changes rather than negative pressure (Fowler Jr. 1945). Aschan (1948) agreed with this possibility and was of the opinion that mechanical factors alone were not the cause of the changes, but that variations in the partial pressure of inspired oxygen beyond certain physiological limits also played a part. He showed that

both decreased pressure and oxygen deficiency gave rise to similar histological changes. However, Dickson, McGibbon and Campbell had shown in 1943 that the changes occurring in otitic barotrauma were not prevented by the administration of oxygen, and most would now agree that although anoxia may occasionally be a contributing factor, it is by no means the most important one in the majority of cases.

The final aspect of otitic barotrauma which merits attention is the phenomenon of "delayed aerotitis". Behnke (1945) considered this due to absorption of oxygen from the middle ear spaces after recompression while breathing oxygen. He postulated that the high oxygen pressure gradient caused diffusion of oxygen out of the air space, and thought that diffusion of nitrogen out of the tissues was too slow to compensate for the oxygen loss. In this way, he felt that a negative pressure may arise over a period of time. In disagreement with this, Chang, Margaria and Gelfan (1950) stated that in their experiments concerning this condition, oxygen absorption in the middle ear did not alter the total volume in the tympanic cavity sufficiently to exceed the change in volume caused by oedema and effusion.

In summary, it would appear that although the artificial and highly negative pressure of clinical and experimental barotrauma may lead to the rapid development of an effusion and mucosal changes, the very much lower negative pressure developed as a result of ventilatory obstruction at atmospheric pressure takes a much longer time to give rise to similar changes and may not even always do so. Secondly, although it is

reasonable that oxygen resorption does occur from the closed pneumatic system, the resultant decrease in pressure is probably progressively compensated by changes such as mucosal oedema, cell blockage and effusion. Admittedly the total size of the pneumatic system in relation to the extent of oxygen resorption and the degree of the "compensatory" mucosal changes must influence the final result, but factors other than oxygen resorption may also be involved in determining the eventual negative pressure and final histological changes.

(b) Effects of ventilatory obstruction on the developing pneumatic system.

In 1926, Brock stated that if the atmospheric pressure effect is cut off, as by chronic catarrh of the Eustachian tube, pneumatisation may be arrested (Brock 1926). This theory of the primary arrest of pneumatisation by ventilatory blockage is difficult to prove clinically. Most cases of Eustachian obstruction are sooner or later associated with pathological inflammatory changes, and for this reason the finding by Murakami and Kudo (1957) that 83% of cases of Eustachian tube stenosis had "arrested pneumatisation" is not necessarily conclusive.

The evidence of certain cases of congenital malformation of the pneumatic system has also been taken to support the concept. Pagenstecher (1958) reported that in severe deformities of the middle ear, solid mastoid bones or bones with severely inhibited pneumatisation are usually seen, and considered that this is at least in part due to insufficient patency of the Eustachian tube or middle ear. However, one must take

into account the possibility that the inhibition of pneumatisation in these cases may be due to the primary action of the same factor which caused the middle ear deformity, and not necessarily to ventilatory blockage as a secondary effect.

The experimental evidence obtained by Opheim (1944) and Greven (1955) has not clarified the problem any further. They found in the chick humerus that pneumatisation is prevented if the foramen pneumaticum is blocked before air cells have begun to develop. However, as has been mentioned already, their procedures not only prevented air from entering the bone, but also interfered with the epithelium at the site of surgical intervention and provided a mechanical obstruction by scar tissue, paraffin or dental cement in addition to causing a mild inflammatory reaction. The experiments of Ojala (1957) are more interesting. After blocking the foramen pneumaticum of some chicks with "orthofil" (a dental acrylic cement), and of others with a free muscle graft shortly after the air space could be detected in the proximal end of the humerus, he found that if the closure was permanent, the formation of the air space was arrested. Although his experiments overcame the objection to interference with the epithelium and its entry into the bone (because the epithelium had already entered the bone), he was still confronted with the development of a slight inflammatory process in the air space. However, as he was also able to demonstrate that slight active inflammation does not arrest pneumatisation per se, he felt justified in concluding that air space development does not continue

in conditions corresponding to those present in longstanding occlusion of the Eustachian tube.

Ojala also reported findings concerning the pathological changes which occurred in the already existing air cells, and described the formation of a dominating zone of reticular new bone inside the cortical bone in the diaphysial area. These changes were found in 50% of his series, and only in chicks over six months of age. He concluded that there may have been a constitutional tendency to reticular bone formation which, promoted by the conditions of ventilatory obstruction, would have finally resulted in extensive sclerotic changes. Unfortunately Ojala did not take the sex of the chicks into account, nor did he mention that he was aware that medullary new bone formation is normal in laying hens and is also seen in oestrogenised cockerels. He therefore appears totally unjustified in making such a conclusion from the available experimental evidence and this is one of the reasons for which it has been considered of value to carry out a more controlled series of experiments on un-caponised cockerels in which the air space of the humerus has reached a stage of partial development, and where the phenomenon of physiological medullary new bone formation is not a complicating factor.

IV MATERIAL AND METHODS.

1. Experimental Animals.

All domestic fowls used in the experimental study were normal, healthy, uncaponised cockerels chosen so that the phenomenon of physiological medullary new bone formation, as demonstrated in two laying hens, might be avoided as a complicating factor when interpreting the pathological changes resulting from the experimental procedures.

A total of 67 male fowls were used, being obtained as follows:-

(i) 22 were of the White Leghorn variety, being supplied by Spillers, Ltd., Middle Aston, Oxon. They were of undertermined age when obtained, but were considered to be at least two months old. They also appeared to be somewhat undernourished, a factor which is known to facilitate the pneumatisation process in chicks.

(ii) 45 were a cross between Rhode Island Red and Light Sussex varieties, the feather markings being a sex-linked characteristic of the breed. The day-old male chicks are brownish in colour, the adult cockerels have distinctive black markings on a white background. These animals were obtained from A.E. Binning, Besselsleigh, Berkshire, in three batches:-

a. the first batch of 20 birds, hatched 3rd June 1964, was obtained when 28 days old.

b. the second batch, hatched 1st July 1964, comprised 15 birds obtained when 42 days old.

c. the third batch comprising 9 chicks of ages varying

from 9-35 days plus one adult cock.

Of this total of 67 birds, 11 died prior to any procedure being performed. The remaining 56 birds were successfully experimented upon.

2. Housing Facilities.

All birds were transferred to and housed at the Research Department of the Nuffield Orthopaedic Centre, Oxford. Two systems of housing were used:-

(i) individual cages which restricted movement and prevented flapping of wings,

(ii) a small, mobile, wooden chicken-house with an attached, totally wire-enclosed run. This allowed full freedom of movement.

In general, the birds were housed in the run prior to operation, kept in individual cages for several days post-operatively, and then returned to the run. However, several particularly large birds were retained in individual cages throughout the entire experiment.

3. General Care.

All birds were vaccinated against Fowl Pest prior to being transferred to the Animal House where they were cared for by the technical staff. For the duration of the experiment, the water and feed hoppers were never allowed to become empty. In this way, a high nutritional standard was maintained. The feed used was Spillers "Intensive Growers Mash C".

4. Anaesthesia.

For the induction and maintenance of general anaesthesia, ether was

administered by the Open method using a conical mask partially filled with cotton-wool into which the beak of the bird was introduced. No supplementary drugs were required and the method proved quite satisfactory.

5. Plain Radiography.

The left wing of each bird was X-rayed pre-operatively, the procedure being repeated at intervals until the stage of pneumatisation was considered to have reached a satisfactory point for the requirements of the experiment. In this way the birds were divided into 2 groups:-

a. those with radiologically complete pneumatisation,

b. those in which pneumatisation was partially complete. For the purposes of the experiment, the stage of pneumatisation preferred was the half way point, but stages between one third to two thirds development were accepted.

To facilitate the taking of X-rays, the smaller birds were held immobile. They remained quiet without an anaesthetic being required. The larger birds needed light anaesthesia. A Watson's X-ray unit was employed, and Ilford "Ilfex" X-ray film was used throughout. The following specifications were complied with in all cases:-

Focal distance - 90 cms.
Voltage - 200 volts.
Current - 15-20 milliamps.
Exposure - $\frac{1}{4}$ sec.

The films were developed in Kodak X-ray developer DI96 for 5 minutes, transferred to Ilford Fixer 22 for 5 minutes, and finally washed in

running water for 20 minutes.

6. Operative Procedures.

The operative procedures were designed to block the anatomical entrance of air to the pneumatic system of the humerus of the domestic fowl, and to create conditions similar to those resulting from obliterative operations as normally carried out on the human temporal bone. The left humerus was used as the experimental bone in most cases, the right humerus being retained as a control.

Each of the two main groups of birds, the fully pneumatised and the partially pneumatised, were divided into two sub-groups. A "Sevriton" block operation was performed on the birds of one sub-group, and a Muscle graft obliteration on the birds of the other sub-group. In this way the following operative groups were established:-

Group I. Fully pneumatised (radiologically).

Sub-group a. "Sevriton" block operation.

Sub-group b. Muscle graft obliteration.

Group II. Partially pneumatised (radiologically).

Sub-group a. "Sevriton" block operation.

Sub-group b. Muscle graft obliteration.

The "Sevriton Simplified" acrylic filling material was provided by the Amalgamated Dental Co., Ltd. It consisted of the following components,

Powder.

a spherical co-polymer of methyl methacrylate, with the addition of sodium fluoride and pigments.

Liquid.

methyl methacrylate, methacrylic acid monomer.

Catalyst system.

this was divided between and incorporated in the powder and the liquid, providing a stable form of sulphinic acid which became active during mixing

(Smith 1964).

The materials were known to produce a dense acrylic mass with a high resistance to distortion under stress (ultimate yield point 803 kg cm^2), a very low solubility factor (0.00035 g cm^2), and a rapid setting time of 40 seconds at 20°C (Amalg. Dental Co.). In addition it was known to have good adhesive properties to bone tissue (McLean, Kramer 1952; Buonocore, Wileman, Brudevold 1956), and to show little shrinkage during polymerisation. Nevertheless, three factors were a potential cause for concern. It was realized that the acid content of the liquid monomer and the generation of exothermic heat during polymerisation might cause an extensive tissue reaction and that the fluoride content may modify osteoblastic activity. However, as it had been stated that the acidity is rapidly reduced following polymerisation, and that the temperature rise with small quantities is between 45-70°C (Smith 1964) and could be kept lower by irrigation (Mahoney 1962), it was decided to test the material, keeping in mind that any tissue reaction would depend largely on the proportions of polymer and monomer used. As will be shown later, the tests demonstrated that the material was satisfactory and accordingly

it was used in the experimental series.

All operations were performed in the Animal House at the Nuffield Orthopaedic Centre, Oxford. General anaesthesia was required in all cases.

Preoperative preparations.

a. The birds were placed on their right side, the left wing being lifted upwards and forwards to reveal the axilla and inner aspect of the humerus.

b. The feathers were clipped from the region of the axilla and from along the length of the humerus.

c. The whole area was then douched with 70% alcohol, draped, and re-douched with alcohol.

d. Sterile operating equipment was used.

Operative procedures.

Two separate operative procedures were used:-

(i) "Sevriton" block operation.

a. A one inch skin incision was made with its centre over the medial tubercle of the proximal end of the humerus.

b. The underlying fascia was dissected to reveal the brachial vessels, the stout tendon of the m. dorsalis scapulae, and the origin of the medial head of the humerotriceps.

c. The tendon was severed close to the bone, and the muscle fibres in and around the foramen pneumaticum were cleared away. In the earlier cases, the tendon was left intact. However, only by cutting the

tendon could a complete view of the foramen pneumaticum be obtained. The step was therefore included in all except the first few birds.

d. The floor of the pneumatic fossa was lightly curetted to ensure clearance of muscle fibres and epithelium and, after bleeding had ceased, it was mopped dry. The walls and the floor of the fossa were then seen to consist of bared, dry bone. In view of the slight over-hang of the periosteum, the cavity was, in effect, an undercut one.

e. "Sevriton simplified" was prepared by placing 8 drops of the fluid monomer in a small glass pot at room temperature, adding the powdered polymer to a dry excess, tapping off the excess powder, and then adding three more drops of the monomer. The mixture was stirred with a rod until it had become viscous. The material was then run into the cavity of the pneumatic fossa and allowed to set. Excess was removed so that the block was level with the periosteum, and adhesion of the block to surrounding muscle fibres was carefully avoided in case this should lead to the pulling out of the block by muscle contraction at a later stage. Setting and adhesion of the block to bone did not seem to be hindered to any significant degree by admixture with a little blood while the "Sevriton" was setting.

f. The wound was sutured with black thread after the block had set hard and solid adhesion to the cavity walls had occurred.

(ii) Muscle graft obliteration.

a. An incision was made from half an inch above to one inch below the medial tubercle of the proximal end of the humerus.

b. The underlying fascia was dissected to reveal the brachial vessels, the stout tendon of the m. dorsalis scapulae, and the proximal half of the medial aspect of the m. biceps brachii and the m. triceps.

c. The brachial vessels were mobilised, the tendon cut (in all except the first few birds of the series), and the medial head of the humerotriceps elevated from its origin from the proximal half of the shaft of the humerus.

d. The floor of the pneumatic fossa was cleared of muscle fibres and removed to expose the interior of the air space of the humerus. The bony cortex around and below the foramen pneumaticum was then removed to create a larger opening into the air space. Finally, the air space was cleared of trabeculae in the region of the opening. In this way the distal two-thirds or so of the bone was left completely untouched.

e. A muscle graft was cut from the medial head of the triceps and pedicled proximally. The graft was then gently tucked into the proximal end of the air space through the large opening previously created.

f. In some cases the graft was anchored to the periosteum or near-by fascia with cat-gut, but in the majority of cases this did not seem necessary.

g. The wound was then sutured with black thread.

Post-operative precautions.

a. All wounds were sprayed with "Nobecutaine". No other

dressing was found necessary.

b. All birds (except the first few) were given one I.M. injection of Penicillin post-operatively. The larger birds were given 500,000 units, the smaller were given 300,000 units.

c. The birds were placed in separate cages for at least 24-48 hours post-operatively. The cages were of a size which prevented excessive flapping of wings during and following the recovery period. By this means it was hoped to avoid excess movement as a factor in the possible dislodgement of the block.

d. All operated wings were inspected at intervals post-operatively. Some haematoma formation was often noted with the muscle graft cases, but no infection was detected and wound healing was excellent.

e. The sutures were not removed. In some cases they had disappeared after a week or two, in other cases they were still present after five to six months without any significant tissue reaction.

Comments.

a. An air leak was often heard when dissecting in the axilla. This was from the opened axillary air sac. It did not appear to cause the animal any respiratory embarrassment and caused no problem.

b. During the performance of the muscle graft operation, an effort was specifically made to identify and avoid the nutrient artery of the humerus.

7. Sacrifice.

To provide a controlled experimental series, the operated birds were

sacrificed at the following specified times:-

a. weekly intervals up to one month.

b. monthly intervals up to eight months.

The first few birds were killed by breaking of the neck. However, this resulted in their passing through a phase of wild and uncontrolled activity prior to death. This activity was thought to be the cause of minor haemorrhage into the air space of the humerus of one bird, and it was also felt likely that there was a risk of fractures occurring because of the chaotic flapping. The method was therefore abandoned and replaced by the simple method of over-dosage with ether.

8. Perfusion Technique: demonstration of blood vessels.

Immediately after death, the thorax and lower region of the neck was quickly opened exposing the heart and great vessels. In performing a right sided arterial perfusion, the right Carotid, right ascending oesophageal, right sterno-clavicular and right thoracic arteries were identified and ligated, (the latter being ligated on the outer surface of the thorax). The right Innominate artery was then ligated as close as possible to the heart and canmulated a little distal to this ligature. The canmula was passed on into the subclavian artery and tied securely. By this means the inflow of perfusion material was largely limited to the right wing itself.

The perfusion technique employed was similar to that previously described by Trueta et al (1947), Trueta and Harrison (1953), Morgan (1959), Trueta and Cavadias (1964), and others. The material consisted

of a well-stirred mixture of the following:-

a. Michrone Berlin Blue (30 gms. in 1,000 cc. water).

b. "Micropaque" - a fine 10% Barium sulphate suspension able to penetrate as far as the capillary bed (Ardran 1953).

These components were mixed in equal proportions.

The perfusion was carried out manually by means of a 20 cc. syringe connected to the cannula with a 6" length of polythene tubing. The perfusion was performed very slowly and gently, avoiding excessive flow. The volume of material used varied between 2-10 ccs. The perfusion pressure was not recorded, as such a recording was not considered to be of value. The adequacy of the perfusion was assessed by consideration of the following factors:-

a. the sensation of back-pressure during the injection,

b. the "bounce-back" of the syringe plunger on release of the pressure of the injecting hand,

c. the appearance of the filling and distension of the vessels in the wing itself,

d. the backflow from the cannula when the injection system was disconnected.

It was considered that the presence of the short length of polythene tubing in the injection system increased the accuracy of the assessment of the first two of these factors.

On completion of the perfusion, the cannula was removed, all the main vessels entering or leaving the wing were tied, and the wing was

removed intact with the scapula attached. An encircling ligature was then placed around the shoulder joint to make doubly certain that no leakage of material could occur, the wing was labelled, and placed in a tank of 10% formalin for 2-5 days.

In the event of arterial studies of both wings being required, the above procedure was repeated on the left side.

In a number of cases, a venous perfusion was carried out. The technique involved was similar to that employed in arterial perfusion in that the corresponding veins were ligated and cannulated in the same manner. The major difference was in the amount of material required. This varied between 10-20 ccs. for complete filling of the venous system of the wing.

To provide additional information concerning the nature and extent of anastomoses occurring within the ulna, several wings were perfused after preliminary ligation of the principal nutrient artery and vein of the ulna.

The exposure of these small vessels was not difficult. An incision was made through the skin of the forewing parallel but just medial to the ulnar artery, commencing proximally at the level of the ulnar recurrent artery and continuing distally for one inch. Then, with care not to injure any vessels, the flexor digitorum superficialis was retracted medially, and the pronator profundus was retracted laterally to reveal the oblique lateral border of the brachialis, parallel to which the nutrient vessels could be seen running towards the ulna. Their passage

through the nutrient foramen could be seen after slight retraction of the flexor digitorum profundus.

As will be mentioned later, the nutrient artery of the ulna may give off an accessory nutrient vessel which passes deep to the brachialis to reach the upper end of the ulna. Care was taken not to include this vessel in the ligature when tying off the principal nutrient artery and vein.

9. Fine Grain Radiography.

The general pattern of the vessels of the intact wing, and the details of the blood supply of the humerus and ulna were studied by means of fine-grain radiography and microradiography. The films were used as an aid in dissection of the vessels of the intact wing, and in the study of Spalteholz preparations of the whole bones.

a. Radiography of the intact wing.

After fixation of the intact wing in 10% formalin for several days, the Micropaque-Berlin Blue mixture became quite hard and did not run (Brookes and Harrison 1957). The scapula was then removed together with the bulk of the muscle tissue around the shoulder joint, and the wing submitted for X-ray studies. For this purpose, a Philips Fine Grain X-ray unit was used, incorporating a Machlett Lab. Type AEG 50 tube which was water cooled at 25lbs. pressure. The following specifications were complied with in all cases:-

Focal distance - 41 cm.
Voltage - 40 Kilovolts.
Current - 15 milliamps.
Exposure - 1 minute 30 seconds.

Kodak Ortho Type 3 Photomechanical Film was employed, and, after exposure, the films were developed in Kodak D-II Fine Grain developer for 2½ minutes at 64°F, transferred to Ilford fixer 22 for 10 minutes, and finally washed in running filtered cold water for 20 minutes.

b. Radiography of whole bones.

The fixed wings were stripped of all soft tissues, and the perfused humerus and ulna were removed with the periosteum intact. The majority of bones were further stripped of their periosteum and all were then decalcified in 5% nitric acid until total removal of calcium salts had been achieved. This process generally took only three days.

The decalcified bones were X-rayed using the same equipment and technique as above except that the exposure used was only 50 seconds. With this method, excellent films of the injected ulnas were obtained, but the injected pneumatised humeri presented a further problem. It was noted that areas of relative radiolucency and radio-opacity were patchily scattered through the bone, thereby impairing clear visualisation of the intra-osseus vessels in all regions. This was shown to be not due to inadequate decalcification nor to a faulty X-ray technique, but to partial filling of the hollow air space with fluid while immersed in the various solutions used for fixation and decalcification. The answer to the problem was to incompletely cut the bone transversely across the shaft after decalcification, bend the bone open and shake out the bulk of the contained fluid. The remainder of the fluid was allowed to evaporate prior to taking the fine-grain X-rays. In this way, much

better films were obtained.

10. Histological Techniques.

a. Fixation.

After excision from the wing and after stripping, all bones were immersed in a relatively large volume of 10% formalin for 2-3 days. They were not split open prior to immersion in the fixative, as this would have involved considerable trauma to the delicate epithelium lining the air space, and would have distorted the general anatomy of the pneumatic system and the intraosseous vessels.

b. Decalcification.

The bones were then transferred to a relatively large volume of 5% nitric acid in water. This was freshly changed every 24 hours until decalcification was complete. The adequacy of the process was assessed by fine-grain radiography, and an average of 3 days was found necessary for total removal of calcium salts.

c. Dehydration.

In order to ensure complete removal of the acid and to initiate the dehydration process, the bones were placed in a relatively large volume of 70% alcohol which was changed three times over a period of 48 hours.

At this stage the bones were cut into proximal and distal halves, the cartilage of the condyles was shaved down a little, and a thin strip of cortex was removed down one face of the shaft. The purpose of these manoeuvres was to facilitate the penetration of the fluids into

and the displacement of air from, the air space.

The bones were then transferred to Absolute alcohol for 36 hours (changed three times), and finally to Benzene for 12 hours (changed twice) to clear the alcohol.

d. Embedding.

It was initially decided to embed the bones in "L.V.N." (Low viscosity nitrocellulose). However, because of the architecture of the air space system and the presence of air, difficulty was experienced with air bubbles persisting inside the bone. Further, a satisfactory section thinner than 12 μ in thickness could not be cut from the L.V.N. blocks and the sections contained large holes (the site of the air bubbles). A change was, therefore, made to Paraffin wax as the embedding substance. This resulted in better impregnation and better displacement of air, and permitted thinner sections to be cut. Both methods are detailed below:-

(1) "L.V.N." Embedding.

After taking the dehydration process to the Absolute alcohol stage, the bone was immersed overnight in a mixture of absolute alcohol and ether (equal parts) and then placed in:-

a. a 5% L.V.N. in alcohol-ether mixture for 5 days,

b. a 10% L.V.N. in alcohol-ether mixture for 4 days,

c. a 20% L.V.N. in alcohol-ether mixture for 2 days.

Following this, the specimen was placed in a mould containing 20% L.V.N., and left for 24 hours in a Dessicater with the lid closed until

all bubbles had risen, thereby giving a bubble-free medium.

By raising the lid of the Dessicator a little, the alcohol and ether were next allowed to evaporate off gradually until a firm block was obtained (if undue evaporation occurred, the L.V.N. was topped up). Occasionally, the hardening process was facilitated by the presence of chloroform vapour, but the controlled slowness of the evaporation was the chief factor in securing a uniformly firm block.

The blocks were then stored in 70% alcohol to prevent further drying off.

(ii) Paraffin wax embedding.

After completion of the dehydration and clearance process as detailed above, the specimen was immersed in Paraffin wax at a constant temperature of 56°C, maintained by keeping the specimen in an incubater. The Paraffin was changed three times over a period of 8 hours to displace the benzene, after which the specimen was embedded.

e. Sectioning.

For this purpose, a Reichert sliding microtome with a wedge-shaped blade was used. The wedge shape of the blade helped to prevent vibration during cutting.

L.V.N. sections were cut at 12 μ and stored in 70% alcohol.

Paraffin sections were cut at 7 μ and placed straight onto albuminised slides.

f. Staining.

The routine stain employed was haematoxylin and eosin, using

Harris's haematoxylin. However, Perl's stain for haemosiderin, an Alcian blue stain for mucin, and Van Gieson's stain for collagen were also employed to obtain further information.

g. Additional techniques.

Two additional techniques were employed in the study and provided information which could not otherwise be obtained.

(i) Frozen sectioning.

Because the histological procedures as detailed above removed all fat from the bones, it was decided to investigate several specimens by blocking them in gelatin and cutting sections with a Freezing microtome after the decalcification, but before the alcohol, stage. These sections were stained with Oil Red O.

(ii) Spaltehols preparations.

To facilitate the study of the vascular anatomy, a selection of perfused ulnas and humeri were rendered transparent by the Spaltehols method after they had been decalcified and X-rayed. This involved the passing the bones through the following fluids:-

a. Hydrogen peroxide 10 vols. for 24 hours.

b. 70% alcohol for 2 days (changed twice).

c. Absolute alcohol for 8-10 days (changed three times).

d. Benzene for 3 days, then changed to fresh Benzene for one week.

e. A solution of equal parts Benzene/Benzyl benzoate for 24 hours.

f. A final solution of 5 parts Methyl salicylate/3 parts Benzyl benzoate.

Full clearing occurred about 2 days after being placed in the final solution. The bones were stored in this solution and examined with the aid of a Zeiss binocular stereoscopic microscope while fully immersed. In certain specimens, selected areas were dissected free for closer examination of the vessels and for mounting on thick slides having a central well. "DePex" mounting medium was used for these and proved quite satisfactory.

V. OBSERVATIONS AND RESULTS.

1. Arterial Supply of the Wing of Gallus Domesticus Proximal to the Carpus.

The literature dealing with the vascular anatomy of birds is now quite extensive, especially with regard to the details of the heart and great vessels (Glenny 1955). However, descriptions of the arterial supply of the avian wing itself are limited, and none of the published works were found to be sufficiently detailed, nor sometimes accurate enough, for the purposes of the present thesis.

The major texts of Stresemann (1927-1932), Grassé (1950), and Marshall (1960) mention only the larger vessels, while those of Sisson and Grossman (1953), and Bradley (1960) give no detail beyond the brachial artery. Better accounts are to be found in the works of Neugebauer (1845), Gadow and Selenka (1891), Kaupp (1918), Otte (1928), Grzimek (1933), Sápy (1941), Fisher (1955), Bhaduri, Biswas and Das (1957) and Westpfahl (1960-61), the latter being the most accurate and most comprehensive regarding the domestic fowl.

In reviewing these, it was notable that there was considerable disagreement between the different authors over the nomenclature of the major vessels, while the accounts of the minor vessels were even more varied. It is certain that much of the confusion has developed because different authors have dealt with different species of birds (Westpfahl 1960-61), but even the nomenclature of the same vessels of the same species, such as Gallus domesticus, varies with the author.

In view of this lack of standardisation, and because of inaccuracies and the total absence of any detail concerning the course and distribution of the vessels supplying the bones of the wing of Gallus domesticus, a detailed study of the whole arterial system of the wing proximal to the carpus was found to be necessary before surgical procedures could be carried out, and before the effects of these procedures could be assessed intelligently.

In the following description, based on combined perfusion, radiological, and dissection studies of 50 wings, the nomenclature used has been derived largely from the previous accounts of Gallus domesticus, although where these have proved inaccurate or insufficiently detailed, I have attempted to supply the most appropriate terminology. It is not intended that any conclusions of phylogenetic significance should be made.

The term "accessory nutrient vessels" should be specifically mentioned. It was first employed by Doan (1922) in relation to small vessels supplying blood to the ends of the radius and ulna of the pigeon. It will be shown later that in the domestic fowl these vessels may supply not only the bone ends, but also the related periosteum, the cartilaginous epiphysis, and the perichondrium, depending on the stage of development of the bone. In the following description it has been found convenient to retain the term as a general one for these vessels.

The terminology with regard to the wing musculature is largely after Berger (1960), but the classic monograph of Hudson and Lanzillotti (1955) has also been followed.

OBSERVATIONS.

The chief vessels observed in the wing of Gallus domesticus proximal to the carpus are listed below, shown radiographically in Fig. 1. and represented diagrammatically in fig. 2.

The Brachial artery.

1. Art. Profunda brachii.
   (i) Humeral artery.
      artery of the pneumatic canal
      nutrient artery of the humerus.
      circumflex artery.
   (ii) descending branch.
   (iii) muscular vessels.
   (iv) propatagial vessels.
   (v) terminal branches.

2. Propatagial branch.

3. Muscular vessels.

4. Ulnar artery.
   (i) propatagial branch.
   (ii) supratrochlear artery.
   (iii) ulnar recurrent artery.
   (iv) posterior metacarpal artery.
   (v) muscular, periosteal and accessory nutrient vessels.

5. Radial artery.
   (i) superficial antebrachial artery.
   (ii) nutrient artery to the ulna.
   (iii) proximal quill artery.
   (iv) nutrient artery to the radius.
   (v) distal quill artery.
   (vi) muscular, periosteal and accessory nutrient vessels.
   (vii) terminal branches.

The Brachial Artery (fig. 3.)

On entering the wing, the brachial artery passes across the posterior aspect of the insertion of the stout tendon of the m. dorsalis scapulae, and across the most proximal fibres of origin of the medial head of the

Fig. 1. Fine grain radiograph of left wing of 14 day old chick.

Fig. 2. Diagram of arteries of right wing of Gallus domesticus proximal to the carpus (Diag. by author).

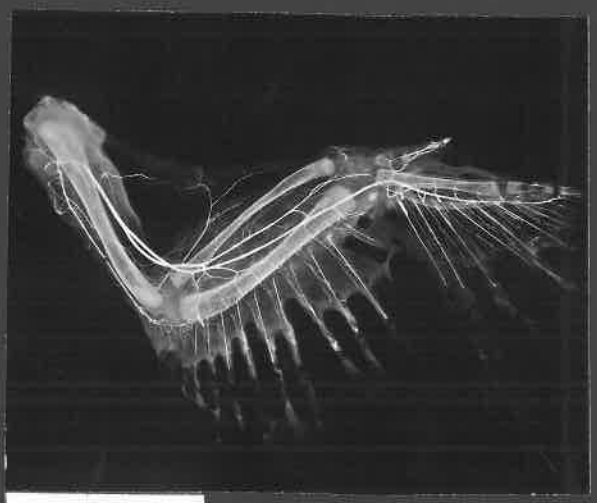


Fig.1

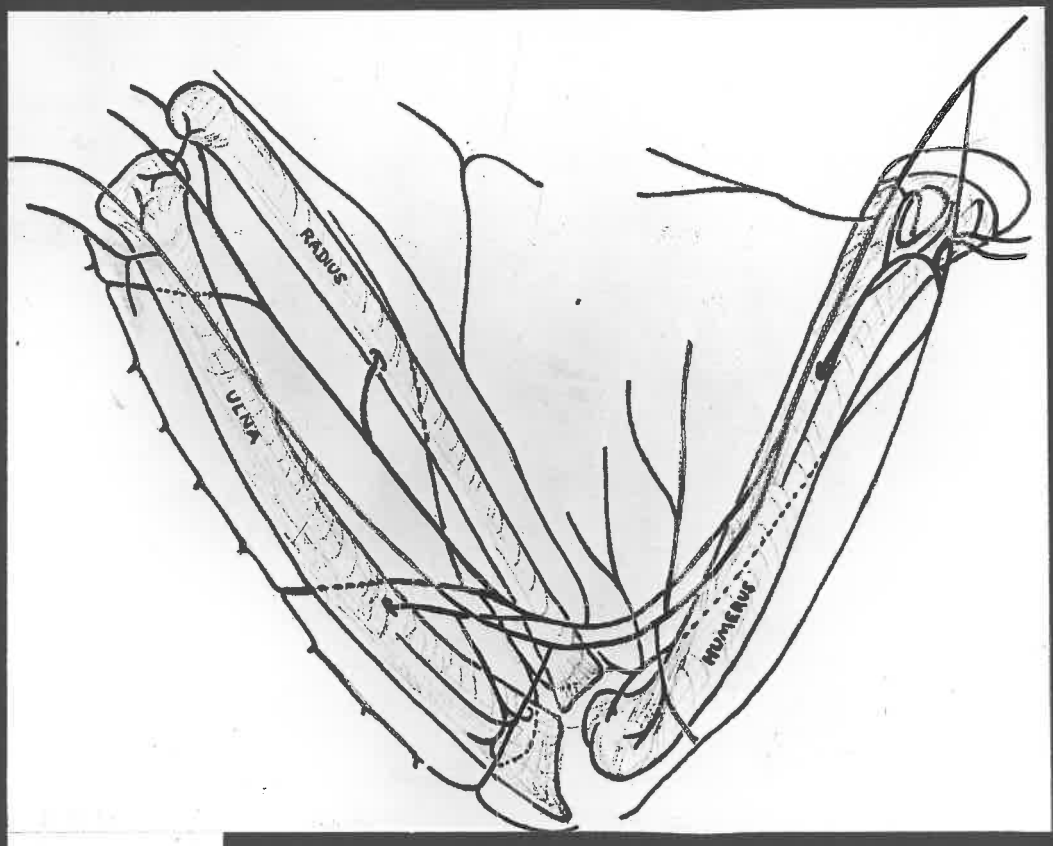


Fig.2

triceps before coming to lie between the m. biceps brachii and the medial head of the triceps. Lower down it lies between the m. biceps brachii and the humerus itself. At a variable point, usually near the midshaft of the humerus, it divides into its two terminal branches, the ulnar artery and the radial artery.

During its course it gives off the following branches:-

1. Art. profunda brachii (figs. 3-5).

This vessel passes distally across the posterior aspect of the tendon of the m. dorsalis scapulae and follows the lateral border of the medial head of the triceps downwards before crossing onto the posterior surface of the lateral head. It then continues between the lateral and long heads of the triceps, winding around the humerus in association with the radial nerve, and passing below the insertion of the latissimus dorsi. It finally emerges on the lateral side of the wing between the triceps and the lower end of the humerus where it divides into its terminal branches. During its course it gives off the following branches:-

(i) Humeral artery (fig 4).

Just distal to the level of the lower margin of the foramen pneumaticum, the art. profunda brachii gives off a short vessel which proceeds antero-laterally towards the V-shaped gap between the origins of the medial and lateral heads of the triceps. The manner of division of this humeral artery is variable. It usually divides into two vessels, the nutrient artery of the humerus and the circumflex artery. However,

Fig. 3. Diagram of ventral aspect of right wing, showing relations of arteries to musculature (Diag. by author).

Fig. 4. Diagram of arteries and musculature in the region of the foramen pneumaticum (Diag. by author).

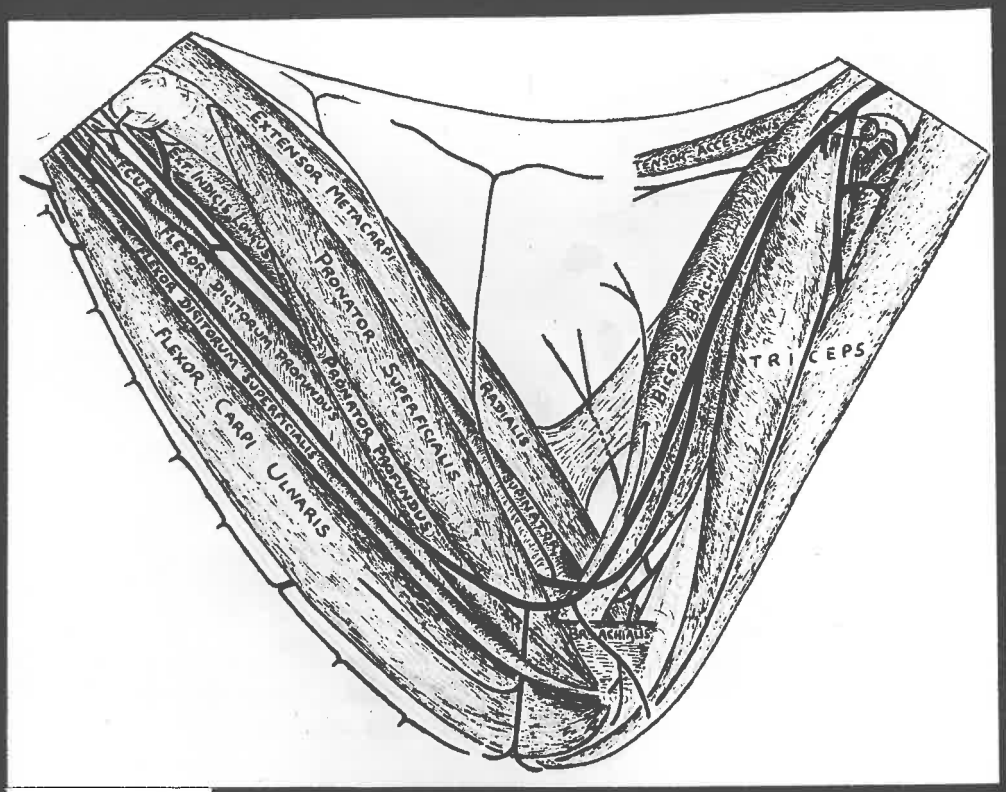

EXTENSOR METACARPI
PRONATOR
SUPERFICIALIS
RADIALIS
BRACHIALIS
TRICEPS
FLEXOR
CARPI
ULNARIS

Fig.3

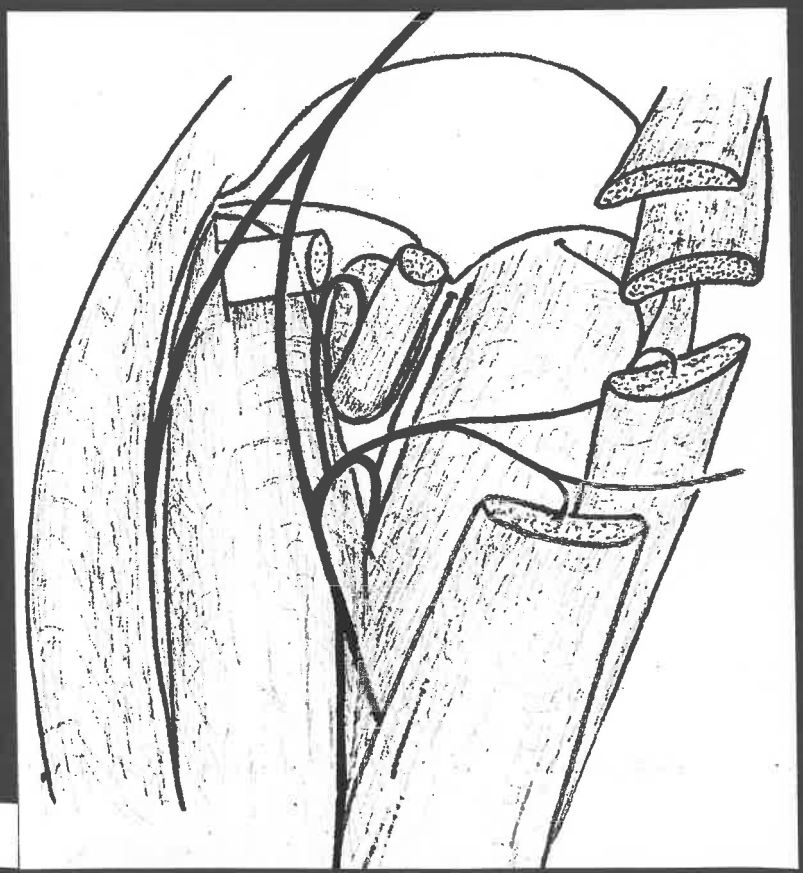


Fig.4

a third artery, the artery of the pneumatic canal, may arise from the humeral artery at or before its point of division, or it may arise from the nutrient artery of the humerus. The picture may be further complicated by another variation. The circumflex artery is normally very short and soon divides into an upper and a lower branch, but in some cases, these branches arise from the humeral artery separately.

The artery of the pneumatic canal (fig. 4) passes upwards along the lateral border of the medial head of the triceps, and, after crossing the posterior aspect of the m. scapulohumeralis anterior (a small muscle which arises from the post-glenoid surface of the scapula and inserts into the pneumatic fossa), skirts the medial margin of the foramen pneumaticum before turning into the pneumatic canal. During its short course it gives muscular twigs to the medial head of the triceps and to the m. scapulohumeralis anterior, and sends a minute accessory nutrient twig across the apex of origin the medial head of the triceps. This passes towards the medial tubercle of the humerus and enters the bone.

The nutrient artery of the humerus (fig.4) passes between the medial and lateral heads of origin of the triceps and continues downwards through the substance of the triceps, gradually approaching the medial border of the shaft of the humerus. At about the midshaft level it enters the nutrient foramen. It sends a minute accessory nutrient vessel proximally along the lateral margin of the foramen pneumaticum before it begins its descent, and muscular twigs, including a long descending one, are given off during its downward course. It should be

noted that in one case of high division of the brachial artery (S.B.7), the nutrient artery of the humerus arose from the Radial artery.

The circumflex artery (fig. 4) is short and continues laterally onto the posterior surface of the lateral head of the triceps where it divides into upper and lower branches. The lower branch passes first between the lateral and long heads of the triceps, then between the long head of the triceps and the m. deltoid major before becoming cutaneous. The upper branch passes between the lateral and long heads of the triceps, and then between the lateral head of the triceps and the deltoid major where it divides into muscular and articular branches. It also sends a small accessory nutrient vessel to the upper end of the humerus.

(ii) Descending branch of a. profunda brachii (fig. 3).

Although it may arise proximal to the humeral artery, the descending branch usually arises from the a. profunda brachii below this point. It continues downwards towards the elbow joint, lying in a superficial position along the posterior surface of the triceps. It supplies twigs to skin and muscle, and takes part in the elbow anastomosis.

(iii) Muscular branches.

In addition to small twigs to the three heads of the triceps, a larger vessel may arise proximal to the humeral artery and assist in the supply of the muscles of the shoulder joint.

(iv) Propatagial vessels (fig. 5).

Just after the a. profunda brachii emerges on the lateral side

of the wing, it gives off one or two small vessels which pass forwards dorsal to the tendon of the tensor patagii brevis to enter the propatagium.

(v) Terminal vessels (fig. 5).

Soon after the propatagial vessels are given off, the profunda brachii generally divides into two terminal branches. The anterior branch is largely expended in supplying the extensor metacarpi radialis, but the posterior branch passes onto the anterior surface of the lower end of the humerus where it divides into muscular, articular, periosteal and accessory nutrient twigs. In addition, one minute twig continues along the radial nerve into the forewing, and another passes to the posterior aspect of the lower end of the humerus.

2. Propatagial branch of the Brachial Artery (fig. 3).

While the brachial artery lies between the m. biceps brachii and the medial head of the triceps, it sends a small vessel through the substance of the m. biceps brachii, supplying the muscle as it goes. The vessel then emerges on the anterior surface of this muscle and follows the lower border of the m. tensor accessorius into the propatagium where it ramifies. In one case of high division of the brachial artery (C.B.7), this vessel was noted to arise from the ulnar artery.

3. Muscular Branches.

The muscular branches which arise f rom the brachial artery are largely confined to the supply of the m. biceps brachii.

4. The Ulnar Artery (fig. 3).

One of the two terminal branches of the brachial artery, the ulnar artery first lies posterior to the m. biceps brachii, but as it proceeds distally, it becomes more superficial and lies on the medial side of the lower end of that muscle. As it continues into the forewing, it crosses superficial to the m. pronator superficialis and m. pronator profundus before coming to lie in a groove between the m. flexor digitorum superficialis and the m. flexor digitorum profundus. These muscles may somewhat overlap the artery in the mid-forewing, but as they narrow towards the carpus, the vessel is clearly seen between their tendons.

During its course to the carpus it gives off the following branches:-

(i) Propatagial branch (fig. 3).

Although this small propatagial vessel may arise from the radial artery or even from the supratrochlear branch of the ulnar artery, it generally arises from the ulnar artery itself and passes forwards either through the substance of the m. biceps brachii or around its medial border to reach its anterior surface. After supplying muscular twigs to the lower half of the m. biceps brachii, it enters the propatagium.

(ii) Supratrochlear artery (fig. 3).

The supratrochlear artery arises from the ulnar artery just before that vessel crosses the m. pronator superficialis. It passes backwards along the upper border of the tendon of origin of that muscle and divides into fine branches which pass over the medial epicondyle to

the posterior aspect of the elbow joint. During its short course it may also give twigs to the anterior surface of the lower end of the humerus. These supply muscular branches to the m. brachialis, in addition to articular, periosteal and lower humeral accessory nutrient vessels. However, these twigs are occasionally derived f rom the radial artery instead of, or in addition to, coming from the supra-trochlear artery.

(iii) Ulnar recurrent artery (fig. 3).

Arising from the ulnar artery as that vessel crosses the m. pronator superficialis, the ulnar recurrent artery passes backwards across the m. pronator superficialis and m. pronator profundus before passing deep to the tendon of origin of the m. flexor digitorum superficialis. While deep to this tendon and the strong fascia which extends ulnarwards from it, the artery crosses the m. entepicondyloulnaris and the m. flexor carpi ulnaris. After giving off a small artery which runs along the surface of the m. flexor carpi ulnaris, the main vessel divides into two branches. One branch passes towards the posterior aspect of the elbow joint and joins in the elbow anastomosis, the other continues to the posterior border of the upper end of the ulna and assists in the supply of the proximal ulnar quills.

(iv) Posterior metacarpal artery (fig. 3).

As the ulnar artery passes across the lower end of the ulna, it gives off a vessel which passes deep to the m. flexor digitorum superficialis and m. flexor carpi ulnaris, to which it gives twigs, and

reaches the posterior border of the lower end of the ulna. It sends off a small branch which assists in the supply of the distal ulnar quills, and winds around the carpus to reach the dorsal aspect of the metacarpus.

(v) Muscular branches, etc.

Numerous branches arise f rom the ulnar artery during its course, most of which supply muscles to which the artery is directly related. At the lower end of the ulna, however, one branch is of further importance in that after passing deep to the m. flexor digitorum profundus it gives periosteal and accessory nutrient twigs to the lower end of the ulna (fig. 3).

5. The Radial Artery (fig. 3).

The other terminal branch of the brachial artery has been the subject of the greatest confusion in the literature. It has been variously termed the "interosseous (radial) artery" (Simons 1960), the "art. radiale eu interosseuse" (Portmann 1950), and the "art. interossea volaris" (Stresemann 1927-1932). However, Neugebauer (1845), Westpfahl (1960-61), and the majority of other workers have referred to it as the "arteria radialis," and this convention is followed here. Nevertheless, it must be pointed out that it does not correspond anatomically with the radial artery of other vertebrates in that it passes into the deep muscle layers of the forewing where it lies in an interosseous position.

The vessel first lies deep to the m. biceps brachii with the ulnar artery, but as it proceeds distally it passes onto the medial side of the tendon of that muscle and enters the forewing by passing through the gap

between the m. pronator superficialis (medially) and the m. supinator (laterally). It continues deeply between the m. pronator profundus and the upper end of the radius, and between the m. pronator profundus and the m. extensor pollicis longus before emerging from under the cover of the m. pronator profundus. It then passes towards the carpus along a groove formed by the adjoining borders of the m. flexor digitorum profundus and the m. extensor indicis longus, and of the m. flexor carpi ulnaris brevis and the m. extensor indicis longus. The vessel ends just proximal to the inferior radio-ulnar joint by dividing into anterior and posterior terminal branches.

During its course to the carpus it gives off the following branches in addition to an occasional propatagial branch, and occasional muscular, periosteal, and accessory nutrient twigs which have already been mentioned:-

(i) Superficial antebrachial artery (fig. 3).

Just before it passes into the forewing, the radial artery gives off a vessel which passes forwards across the supinator to reach the ventral aspect of the m. extensor metacarpi radialis. Here it divides into two main branches. One continues along the m. extensor metacarpi radialis towards the carpus, giving off small muscular and propatagial vessels as it goes, the other crosses the m. extensor metacarpi radialis obliquely to enter the propatagium, at the free margin of which it divides into ascending and descending vessels. The superficial antebrachial artery, which has been given various names by various authors, was

regarded as the true radial artery by Gadow and Selenka (1891), and, from its position, this seems reasonable.

(ii) Nutrient artery to the Ulna (fig. 2).

While lying deep to the m. pronator profundus, the radial artery gives off a vessel which crosses the ventral aspect of the m. extensor pollicis longus to reach the oblique lateral border of the m. brachialis. It follows this border towards the ulna before passing deep to the origin of the m. flexor digitorum profundus and entering the ulnar nutrient canal. During its course, the nutrient artery gives off a vessel which passes deep to the m. brachialis, curves around onto the medial surface of the upper end of the ulna, and supplies periosteal and accessory nutrient twigs to this aspect of the bone. It should be noted, however, that this tiny twig may arise from the radial artery itself. The nutrient artery of the ulna also gives off periosteal and muscular vessels, including one which crosses the m. brachialis superficially to supply the m. entepicondylo-ulnaris.

(iii) Proximal quill artery (fig. 5).

Arising with or just distal to the nutrient artery to the ulna and passing backwards between the upper end of the radius and the proximal fibres of origin of the m. extensor pollicis longus, this vessel crosses the free border of the m. anconeus and continues between the m. anconeus and its two overlying muscles, the m. extensor digitorum communis and the m. extensor carpi ulnaris. After emerging between the ulnar borders of the m. anconeus and the m. extensor carpi ulnaris, it

Fig. 5. Diagram of dorsal aspect of right wing, showing relations of arteries to musculature (Diag. by author).

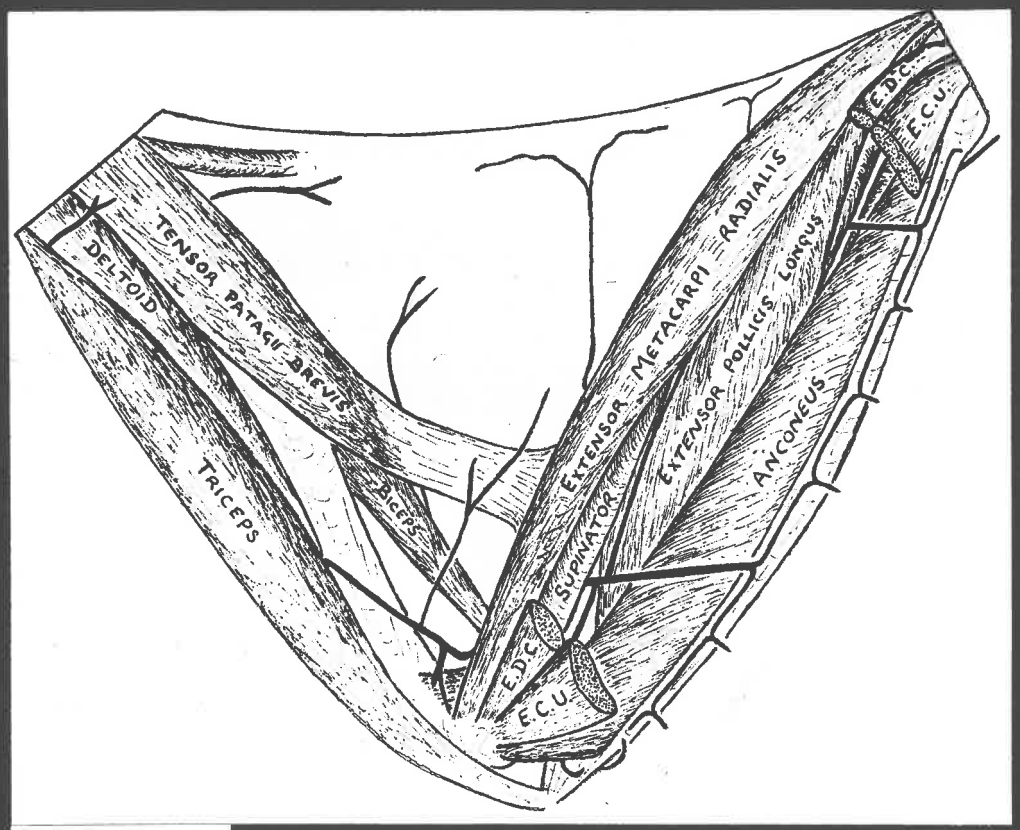

TENSOR FASCIÆ BREVIS
DELTOID
TRICEPS
BICEPS
EXTENSOR METACARPI RADIALIS
SUPINATOR
EXTENSOR POLLICIS LONGUS
ANCONEUS
E.D.C.
E.C.U.
E.C.U.
E.D.C.

Fig.5

divides into ascending and descending branches. These give off small vessels at right angles to supply the ulnar quills. The descending branch anastomoses with the ascending branch of the distal quill artery which will be described later.

Apart from several muscular twigs, the proximal quill artery gives off two fairly constant branches during its course. Both arise before the parent vessel crosses the m. anconeus. The first runs proximally towards the lateral aspect of the head of the radius and gives off a branch which passes deep to the m. anconeus to reach the lateral aspect of the upper end of the ulna. Muscular, periosteal, and accessory nutrient twigs arise from these small vessels. The second runs distally between the m. supinator and the m. extensor digitorum communis before becoming superficial between the m. extensor metacarpi radialis and the m. extensor digitorum communis. It gives rise to muscular and cutaneous twigs.

(iv) Nutrient artery to the Radius (fig. 2).

At about the middle of the forewing, the radial artery gives off a small vessel which crosses the ventral aspect of the origin of the m. extensor indicis longus before entering the nutrient foramen of the radius. It gives off small periosteal twigs prior to entering the bone.

(v) Distal quill artery (fig. 5).

While lying in the groove between the borders of the m. extensor indicis longus and the m. flexor carpi ulnaris brevis, the radial artery gives off a vessel which passes backwards between the m. extensor

indicis longus and the most distal fibres of the m. anconeus. It crosses the m. anconeus and the lower end of the ulna while lying deep to the m. extensor digitorum communis and the m. extensor carpi ulnaris, and finally appears between the latter muscle and the lower end of the ulna. Here it divides into ascending and descending branches which give off small vessels at right angles to supply the ulnar quills. The ascending branch anastomoses with the descending branch of the proximal quill artery.

(vi) Muscular branches etc.

In addition to those branches already mentioned, minor muscular and periosteal vessels are given off during the course of the Radial artery.

(vii) Terminal branches (fig. 3).

Just proximal to the inferior radio-ulnar joint, the radial artery divides into anterior and posterior terminal branches. The anterior branch gives periosteal and accessory nutrient twigs to the lower ends of both radius and ulna and continues onto the ventral aspect of the carpus. The posterior branch runs across the m. extensor indicis longus, and then passes backwards between this muscle and the lower end of the radius. It continues onto the posterior aspect of the carpus.

2. The Intraosseous vasculature of the ulna and humerus of Gallus domesticus.

In view of the absence of information concerning the basic pattern of the intraosseous vessels of the long bones of Gallus domesticus, and, in particular, the absence of any information concerning the blood supply of the avian humerus at various stages of pneumatisation, a detailed investigation of these points was necessary in order to assess the histological and vascular effects of the proposed surgical procedures on the chick humerus. However, to compare the effect of pneumatisation on the vascular pattern of the chick humerus with the pattern existing in a chick bone which is permanently narrow containing, the arrangement of the intraosseous blood vessels of such a bone at various stages of development had to be established. The chick ulna was chosen for the study.

60 wings were used. The ages varied from 9 days to approximately 12 months, and wings of both right and left sides were studied. After sacrifice, the wings were injected and disarticulated for fixation. The bones were X-rayed after decalcification and cleared by the Spalteholz technique. The specimens were then examined whole, and areas of importance were dissected for more detailed study.

a) Observations on the ulna.

1. The arterial supply.

(i) The nutrient artery.

The shaft is chiefly supplied by the nutrient artery which, on reaching the junction of the middle and upper thirds of the bone, passes

through the bony cortex at an oblique angle directed distally, and enters the marrow (Fig. 6). This site and direction of entry was found in all except five of the ulnas examined. In three the nutrient artery penetrated the cortex at right angles at the midshaft level, in the other two it was sited at the junction of the middle and lower thirds of the bone and was directed proximally (Fig. 7).

Lying just beneath the cortex, the artery continues its oblique course distally for a short distance before dividing into ascending and descending divisions. No minor branches are given off within the shaft of the bone before this major division occurs.

The ascending division often continues distally with the descending division for a short distance before deviating centrally and curving back through almost 180° to pass along the centre of the shaft in a proximal direction. At a variable point, often about the level of the nutrient foramen, it divides into ascending branches. In general two main ascending branches may be seen which divide and subdivide as they continue towards the proximal end of the bone. However, both the level of division and the apparent number of main branches vary. During its course, the perfused ascending division may appear somewhat tortuous.

The descending division proceeds obliquely onwards as a direct continuation of the main nutrient trunk to reach the centre of the shaft a short distance below the nutrient foramen. It then continues distally along the centre of the shaft and at a variable level, often near the junction of the middle and distal thirds of the bone, divides into

descending branches. In general, two main descending branches may be seen which continue towards the distal end of the bone, dividing into further sub-branches as they go. The pattern is more constant, and the vessels tend to be straighter compared with the ascending division, but variations are frequent.

The pattern of branching and the general course of the side vessels which arise from the two divisions and their main branches is such that three groups of minor vessels can be described (Fig. 8):-

1) vessels which arise at an acute angle and pass obliquely along the shaft for a variable distance in the direction of the parent vessel before turning towards the cortex, branching as they go. These do not reach the bone ends.

2) short vessels which arise more or less at right angles from the parent vessel, pass radially outwards, branching as they go, and fan out as they reach the endosteal surface of the cortex. They are few in number, generally restricted to the central section of the shaft, and are chiefly seen in the older rather than the very young bones. They probably represent the more centrally originating oblique vessels whose course has been relatively altered by differences in the growth rate of the shaft in relation to the growing arterial supply.

3) vessels which arise by repeated division and subdivision of the main branches of the nutrient artery, forming a spray of vessels which supply the growing bone ends. From this spray, which becomes very dense in the spongy zones, individual vascular loops are seen which invade the

Fig. 6. 14 day old chick ulna. Arterial perfusion showing normal entry of nutrient artery and course of its branches. Radiograph.

Fig. 7. 35 day old chick ulna. Arterial perfusion showing proximally directed nutrient artery and its branches. Radiograph.

Fig. 8. Ulna. Arterial perfusion showing pattern of branching of nutrient artery system. Bone growth has almost ceased. Radiograph.

Fig. 9. Ulna. Invasion of the metaphyseal aspect of the growth plate by terminal vessels of the nutrient artery system. Perfused specimen. H & E x 100.

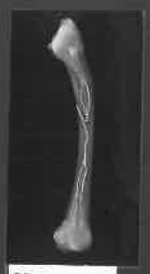


Fig.6

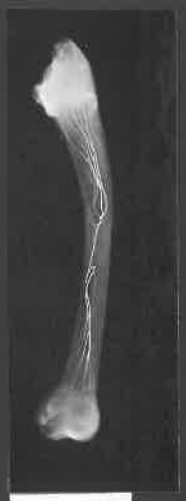


Fig.7

Fig.8

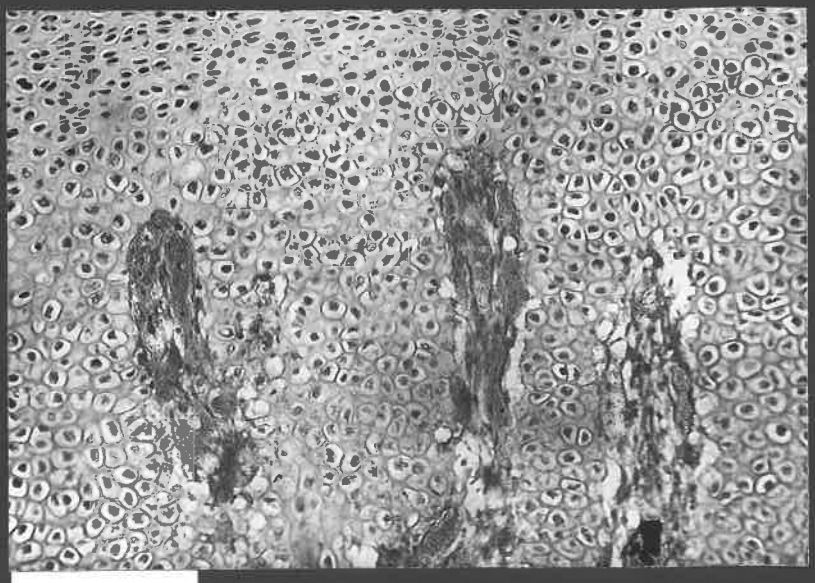


Fig.9

metaphyseal aspect of the growth cartilage (Fig. 9) after the manner previously described. The pattern of these loops is interesting. In injected specimens some of them appear as simple loops by virtue of the outgoing vessel turning sharply back on itself. However, others are more complex. At its extremity the outgoing vessel is seen to break into several sinusoidal channels which immediately pass backwards and ensheathe the principal vessel, giving rise to a bulbous, basket-like pattern (Fig. 10).

When the growth plate has been exhausted and the invasion of the hyaline zone of cartilage has been completed, these vessels are no longer seen (Fig. 11).

(ii) Arteries of the bone ends.

As previously mentioned, there are no centres of epiphyseal ossification in the long bones of the chick wing, and up to about 155 days the cartilaginous ends present three zones, a narrow outer zone of articular cartilage, a wide middle zone of hyaline cartilage, and an inner zone of growth cartilage.

The middle hyaline zone is sparsely penetrated by vessels, some passing directly inwards from the perichondrial vessels, the majority entering the peripheral region of the cartilage near its junction with the bone at the point where the perichondrial and periosteal vessels communicate (Fig. 12). These vessels pass along the so-called cartilage canals and tend to run an arching course in conformity with the convexity of the growth plate. They give rise to few branches and do not

Fig. 10. Basket-like pattern of a terminal vascular loop of the nutrient artery system of the ulna.
Spalteholz preparation x 200.

Fig. 11. Fully grown ulna. Arterial perfusion. All that remains of the cartilaginous epiphysis is the articular cartilage. The terminal nutrient spray of vessels is no longer seen.
Radiograph.

Fig. 12. Young ulna, showing the arching vessels of the cartilaginous epiphysis. Spalteholz preparation x 16.

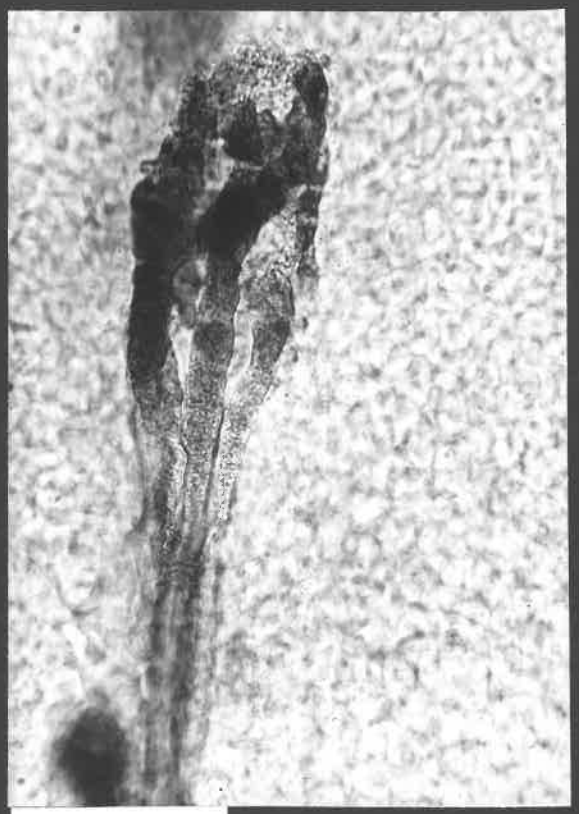


Fig.10

Fig.11

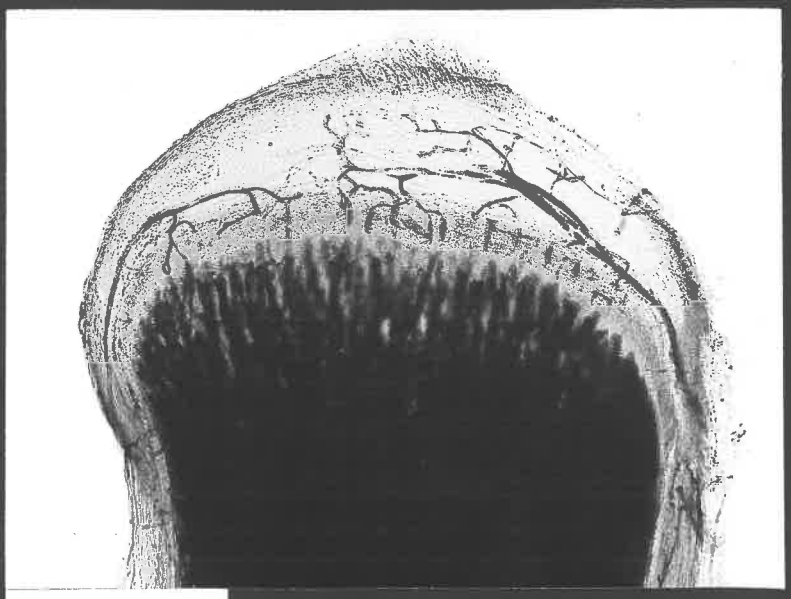


Fig.12

anastomose with each other. Further, in young bones they are relatively more closely spaced compared with the wider spacing seen in adult bones.

Those which arch close to the epiphyseal surface of the growth cartilage are of special interest, because not only do they maintain this close relationship until the stage of exhaustion of the growth zone, but they also send occasional blood vessel loops vertically into this zone (Fig. 13). Although some of these loops may appear to pass through the entire thickness of the growth plate, the majority do not and, in the material examined, no communication with the diaphyseal vessels was observed at any stage. The vessels of the cartilaginous epiphysis are nevertheless linked extraosseously with those of the metaphyseal region by virtue of their external connections with the communicating system of perichondrial and periosteal vessels.

The loops of the cartilage vessels, both those ending in the hyaline zone and those partially penetrating the growth cartilage, present features similar to the pattern described by Trueta (1957) in the cartilaginous epiphysis of the human femoral head. They exhibit a bulbous form by virtue of the artery breaking into a number of capillaries which surround the termination of the artery and then join into a single vein which runs back along the cartilage canal.

Another striking feature is that the cartilage canals are surrounded by proliferating cartilage cells developing matrix (Figs. 14a, 14b). This fact, together with the more commonly accepted theory of the interstitial growth of cartilage, probably largely accounts for the

Fig. 13. Young ulna. Vascular loops enter the growth plate from its epiphyseal aspect but do not pass right through. Spaltehol2 preparation x 25.

Figs. 14(a), 14(b). Sections through the cartilaginous epiphysis of a young ulna showing a cartilage canal. The artery contains perfusion material. Single cartilage cells are found in the immediate vicinity of the vascular bundle, but farther away they appear first in pairs, and then in groups of four. H & E x 200.

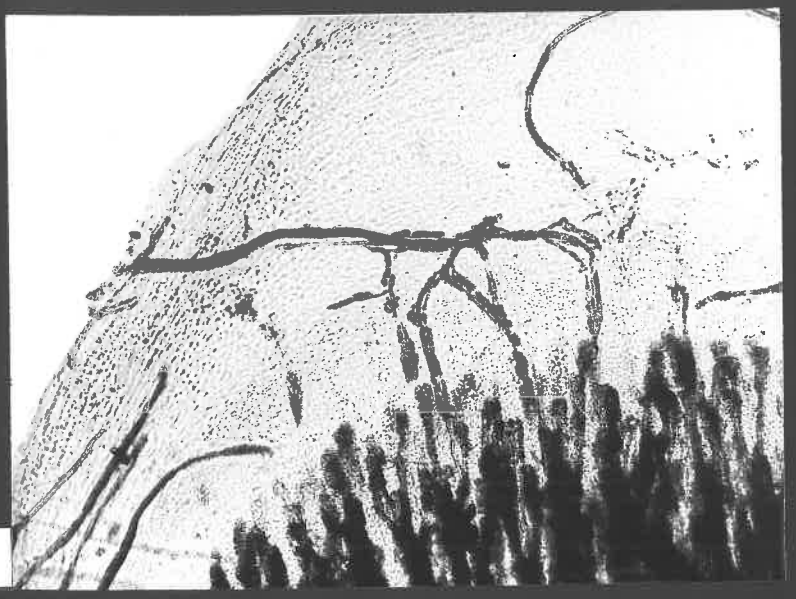


Fig.13

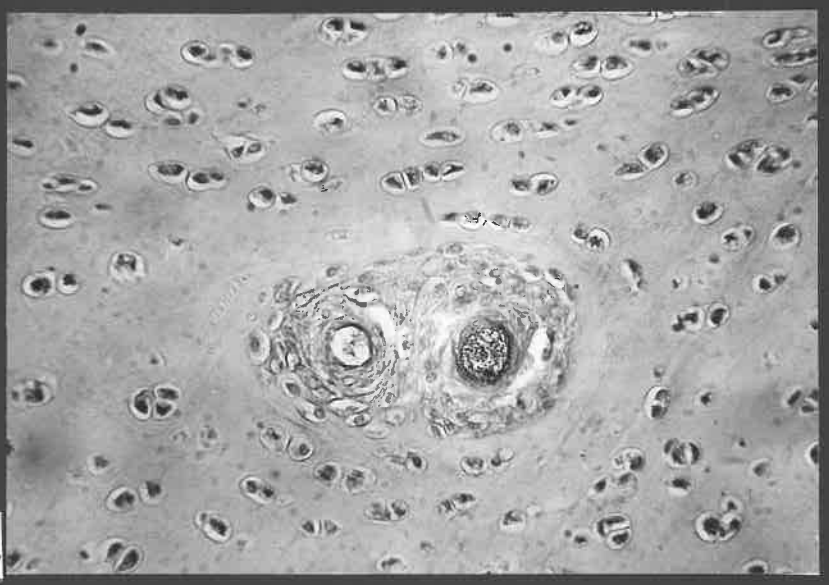


Fig.14a

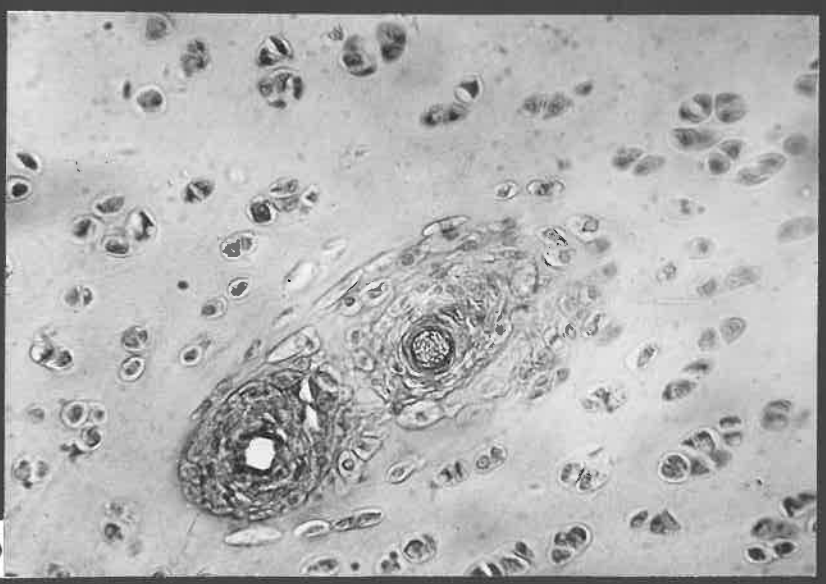


Fig.14b

increase in bulk of the cartilaginous epiphysis with age, and explains the widening of the distance between individual canals previously mentioned. However, whether the cartilage cells in the immediate vicinity of the canals proliferate preferentially by virtue of their proximity to the blood supply conveyed by the canals, or whether the cartilage cells are actually derived from the proliferation and differentiation of the mesenchymal cells surrounding the vessels in the canals could not be determined.

With the exhaustion of the cells of the growth cartilage, the brush-like terminal loops of the nutrient artery system, and the marrow itself, start to invade the hyaline zone. The sparse vessels of this zone are slowly enveloped by the advancing front and become incorporated in the blood supply of the terminal region of the endochondral zone of bone (Fig. 15). They initially maintain their arcuate course, but this may become considerably modified with further growth. In addition, they markedly increase their area of ramification. Small branches pass towards the terminal plate of bone, subdividing to reinforce the vascular network at the bone end, while others proceed centrally (Fig. 16). These latter vessels develop direct anastomoses with the branches of the nutrient artery (Fig. 17), a feature confirmed by retrograde filling of the main nutrient system of the adult ulna via these vessels after ligation and division of the nutrient artery and vein, combined with periosteal stripping. Together with the metaphyseal vessels, the arcuate vessels appear to largely take over the blood supply of the bone

Fig. 15. Stage of closure of the growth plate. The vessels of the hyaline zone are becoming enveloped by the advancing diaphyseal vessels. Spaltehol z preparation x 20.

Fig. 16. Fully grown ulna, showing the arcuate artery. This vessel originally supplied the cartilaginous epiphysis. Spaltehol z preparation x 16.

Fig. 17. Fully grown ulna. The ramifications of the arcuate artery are shown. An anastomosis with a branch of the nutrient artery is seen. Spaltehol z preparation x 16.

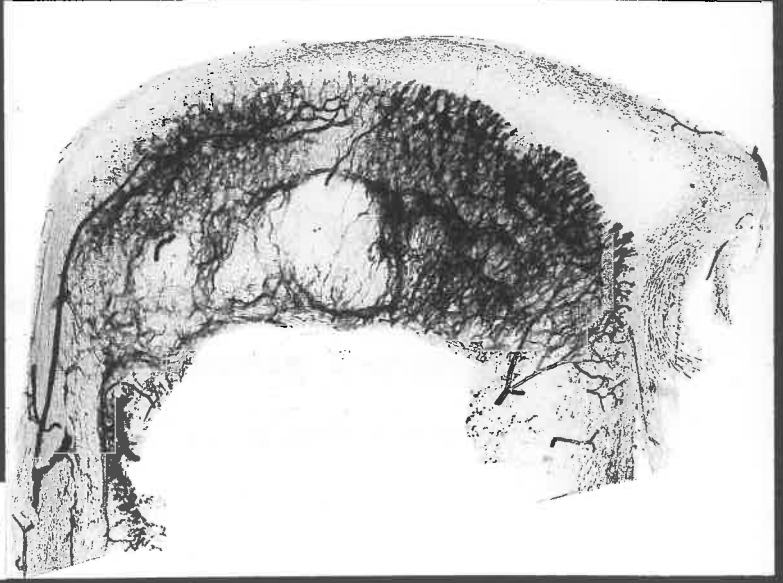


Fig.15

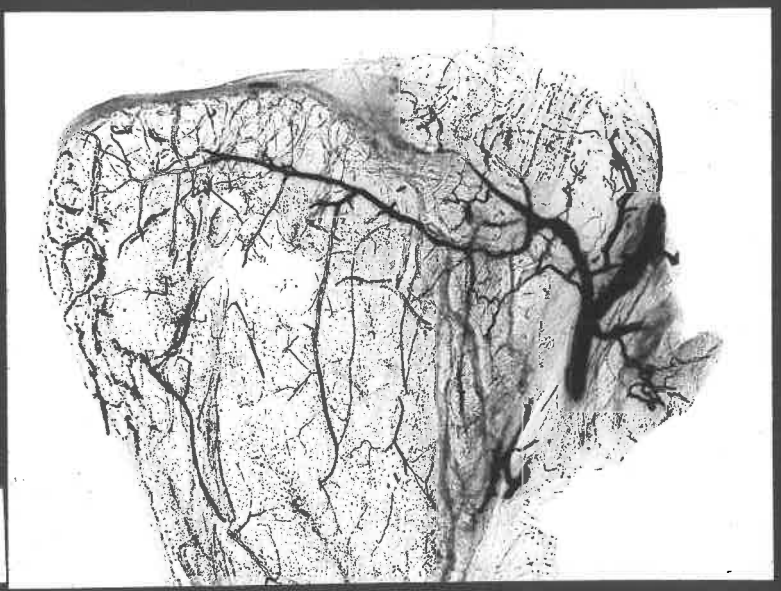


Fig.16

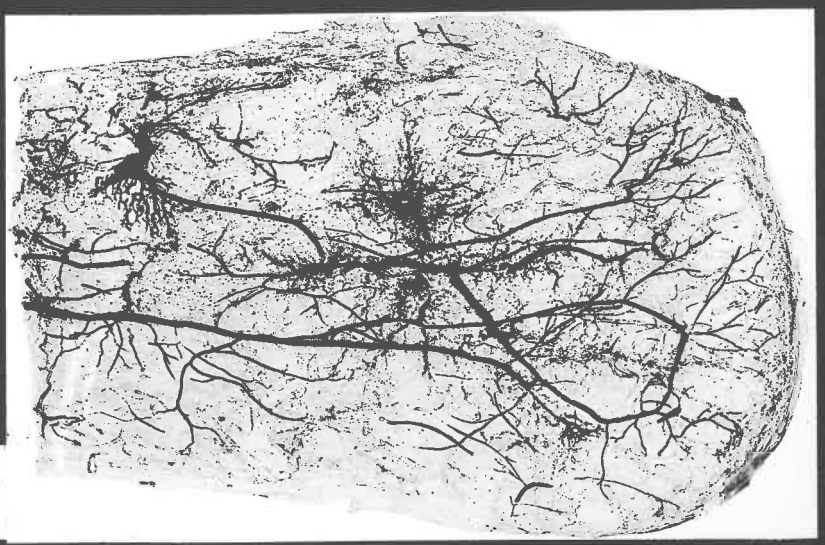


Fig.17

ends in the adult (Fig. 18).

(iii) Periosteal and Cortical vessels.

In addition to small vessels which arise from the nutrient artery before it enters the bone, the periosteal network is supplied by numerous small vessels along the length of the shaft. The larger of these are at the bone ends, especially the proximal end. As they approach the periosteum, they give off numerous branches which spread out at random, subdividing and anastomosing to form an open-laced pattern (Fig. 19).

The shaft cortex is honeycombed by a complex system of vascular channels which run more or less parallel to the bone cortex in the mid-shaft region, but incline obliquely outwards and away from the midshaft in other regions (Fig. 20). This is in conformity with the diverging direction of the branches of the nutrient artery as the bone ends are approached. These channels are linked together by others running at right angles to them.

The cortical system communicates superficially with the periosteal vessels, and at its medullary surface is connected with the branches of the nutrient artery. Occasional vessels derived in some cases from the periosteal arteries and in others from the nutrient branches tend to pursue a distinct course, sometimes direct, sometimes devious, through portions of the cortex rather than immediately dispersing into the lattice-like system of vascular channels. The direction of arterial flow through this system was a problem beyond the scope of the present

Fig. 18. Adult ulna, partial arterial perfusion. By differential perfusion it is possible to show that the arcuate vessels tend to take over the blood supply of the bone ends. Radiograph.

Fig. 19. Young ulna. Arterial perfusion. The periosteal vessels are superimposed on the nutrient system. Radiograph.

Fig. 20. Ulna. Venous perfusion. Spalteholz preparation of the mid-shaft region showing varying direction of the vascular channels in the cortex x 16.

Fig. 21. Ulna. Spalteholz preparation showing the one third/two third distribution of the cortical vessels x 36.

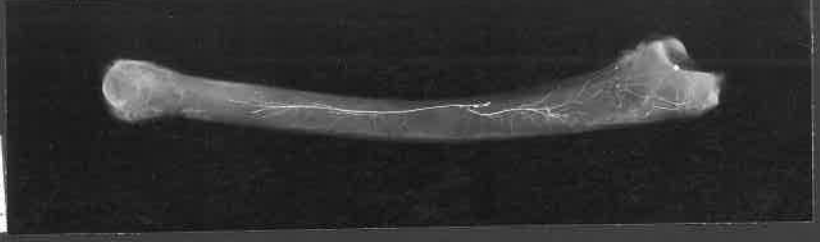


Fig.18

Fig.19

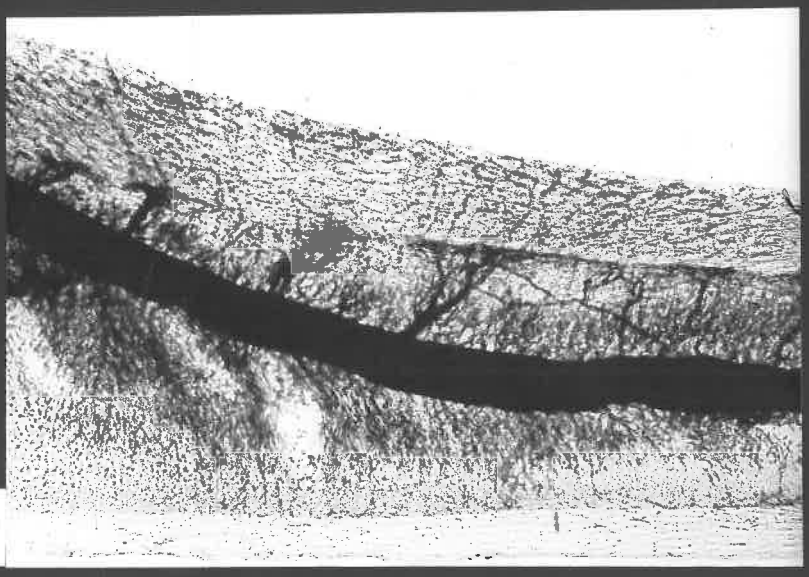


Fig.20

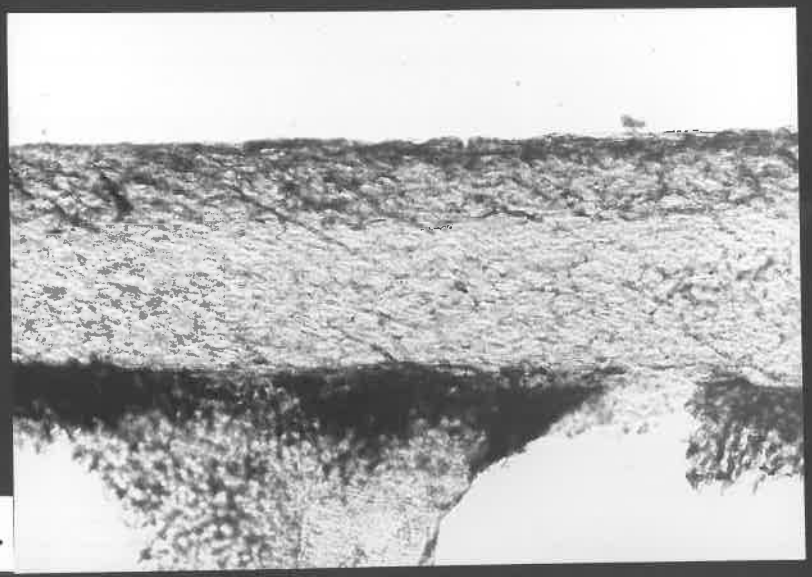


Fig.21

investigation, but it was noticeable that the outer third of the system filled more densely on perfusion (Fig. 21), and that the channels here appeared to be more closely packed than in the inner two thirds.

At the shaft extremities, occasional periosteal vessels were seen to assume the role of true metaphyseal arteries, passing directly through the cortex and breaking into branches which proceeded towards the peripheral fringe of the metaphyseal aspect of the growth plate. No difference was found between the end vessels of these metaphyseal arteries and those of the nutrient artery. However, it was interesting that they could not be demonstrated in the younger chick ulnas, suggesting that they were established at some intermediate stage prior to the exhaustion of the growth plate.

2. The venous system.

(i) The central medullary vein.

The medullary vein actually consists of centrally placed ascending (distal) and descending (proximal) portions which unite a little proximal to the midshaft level to form one common efferent vein. This passes obliquely out of the bone through the nutrient foramen. The ascending portion passes directly into the common efferent vein, whereas the descending portion characteristically deviates a little posteriorly before looping around to join the ascending portion at a little less than a right angle (Fig. 22).

Each of the two divisions is formed by the union of two main tributaries which are in turn formed by the joining of lesser tributaries.

The level of the main union is interesting. In young bones up to 14 days, it may correspond with the level of the primary splitting of the divisions of the nutrient artery into branches. In more mature bones, the union is invariably closer to the bone end than the level of primary arterial branching, indicating a difference in growth rate of the various elements of the arterial and venous systems within the developing bone.

The pattern of the tributaries draining into this basic system is quite different f rom the pattern of arterial division within the bone. The primary units are the extensively distributed, thin-walled venous sinusoids, and three patterns of drainage of these vessels into all sections of the central vein may be seen along the shaft of the bone (Fig. 23):-

a) sinusoids arising close to the central medullary vein drain directly into the vein itself, or its tributaries, giving it a rather feathery appearance.

b) groups of sinusoids which drain areas a little farther away from the central vein tend to channel into small collecting vessels which in turn empty directly into the central vein or its tributaries. This gives rise to a somewhat tufted appearance.

c) groups of sinusoids from the peripheral regions of the medullary cavity and f rom the growing bone ends first drain into small collecting vessels, and these join with other similar vessels to form a branch-like pattern, the stem of which joins the great vein or its tributaries.

Except at the bone ends, the sinusoids, or their collecting vessels

enter the principal vein at right angles, in marked contrast to the pattern of branching of the arterial system.

(ii) Veins of the bone ends.

These follow their corresponding arteries closely so that in the cartilaginous epiphysis, the cartilage canals contain both arteries and veins. No sinusoids are seen and there is no drainage across the growth plate to the medullary venous system. Drainage occurs chiefly towards the junctional region between perichondrial and periosteal veins.

With the exhaustion of the growth cartilage and the invasion of the hyaline zone by the diaphyseal vessels, the veins of the cartilaginous ends become incorporated into the venous drainage of the bone ends in a similar manner to the incorporation of the corresponding arteries (Fig. 24.) They may initially be seen as arcuate or transversely running vessels, but their course may be considerably modified with further growth (Fig. 25). However, their tributaries at this stage assume a sinusoidal appearance, and a linkage occurs with the central vein system via the sinusoidal network rather than by the direct anastomosis of larger channels.

(iii) Periosteal and cortical veins.

The system of periosteal veins, including the presence of metaphyseal veins, which are generally more numerous than their corresponding arteries, closely follows the system of arteries. The venous pathways in the lattice-like system of channels of the shaft cortex likewise communicate with the periosteal veins superficially and with the sinusoids

Fig. 22. Venous perfusion showing the general pattern of the medullary vein. Radiograph.

Fig. 23. Ulna. Medullary vein and its tributaries. Spalteholz preparation x 25.

Fig. 24. Ulna. Arcuate vein and its sinusoidal tributaries. Spalteholz preparation x 16.

Fig. 25. Adult ulna. Venous perfusion. The course of the arcuate veins is often considerably modified by the final stages of bone growth. Radiograph.

Fig.22

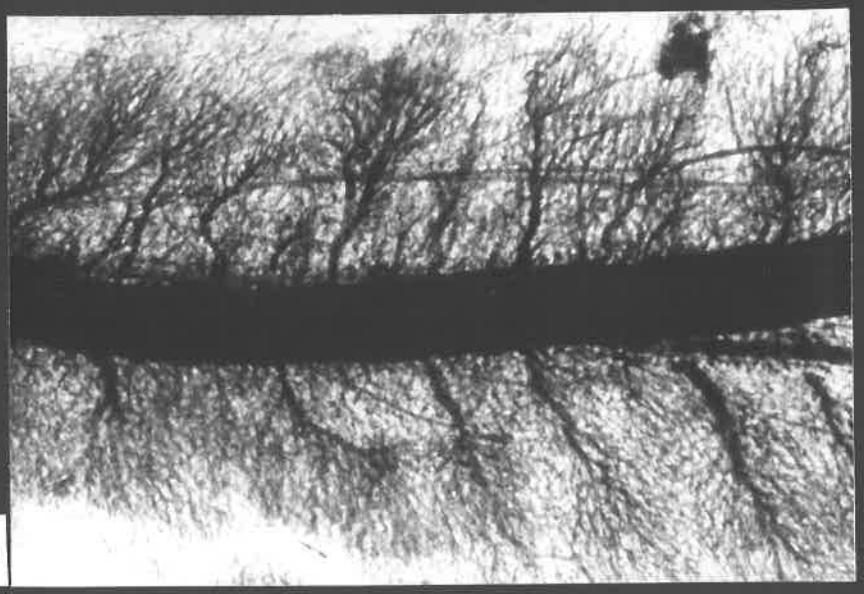


Fig.23

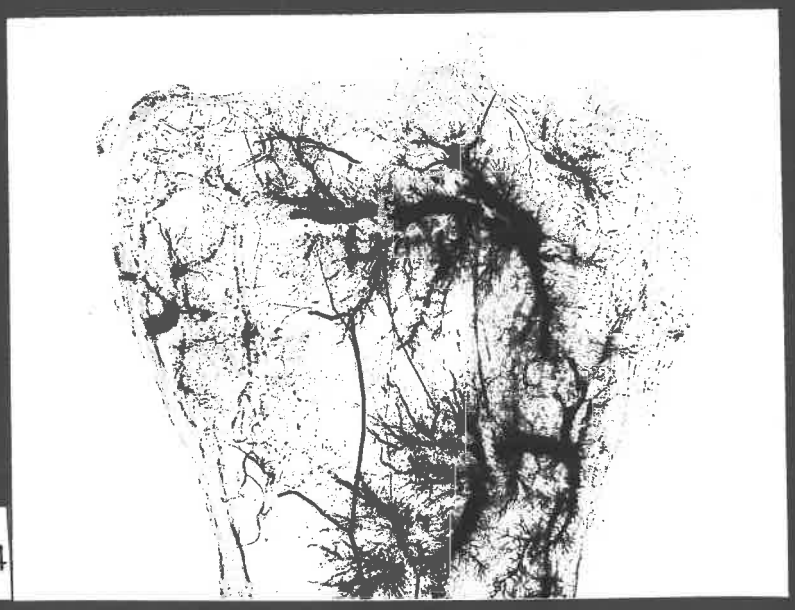


Fig.24

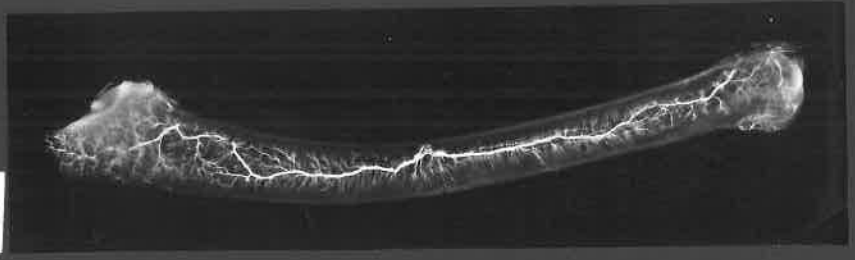


Fig.25

of the peripheral areas of the marrow on the endosteal side of the cortex.

b) Observations on the chick humerus.

1. Vascular pattern prior to pneumatisation.

The intraosseous arrangement of both arterial and venous systems in the shaft and cartilaginous ends of the chick humerus prior to pneumatisation is basically similar to that of the chick ulna at the equivalent stage of development. The distribution of the nutrient artery (Fig. 26), the sinusoidal drainage pattern into the medullary vein (Figs. 27a, 27b), the periosteal vessels (Fig. 28) and those of the cartilaginous ends appear similar in all respects. In all the humeri examined, the nutrient canal was directed distally and was situated a little more towards the midshaft level than in the ulna.

There is, however, one outstanding exception to this generalisation of similarity between the two bones. In the upper third of the humerus a large number of sinusoids drain into three, or sometimes more, transversely or obliquely running collecting vessels (Figs. 29a, 29b). These pass medially and enter a longitudinally running vein, sometimes duplicated, in the position of the future pneumatic canal. This vein passes out of the bone at the site of the future pneumatic foramen, and the entire arrangement is superimposed on the general pattern of medullary venous drainage of the proximal end of the bone.

There is no intraosseous artery corresponding to this proximally draining vein. The future artery of the pneumatic canal may be seen

Fig. 26. 14 day old humerus. Arterial perfusion. The distribution of the nutrient artery in this bone is similar to that in the ulna. Radiograph.

Fig. 27(a), 27(b) 14 day old humerus (27a) and 28 day old humerus (27b). The drainage pattern of the medullary vein is similar to that in the ulna except at the proximal end of the bone. Radiographs.

Fig. 28. 35 day old humerus. Arterial perfusion. The periosteal vascular pattern is superimposed on the pattern of the nutrient system. Radiograph.

Figs. 29(a), 29(b). Young humeri prior to pneumatisation. In the upper third of the bones, an additional venous system is seen. This drains through the future foramen pneumaticum. Spaltenholz preparations x 16 (29a), x 20 (29b).

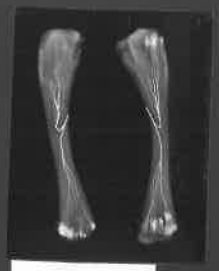


Fig.26

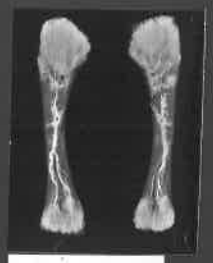


Fig.27a

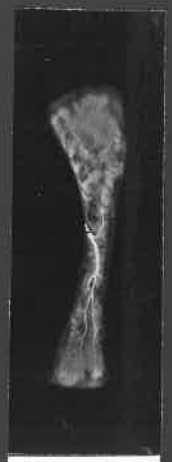


Fig.27b

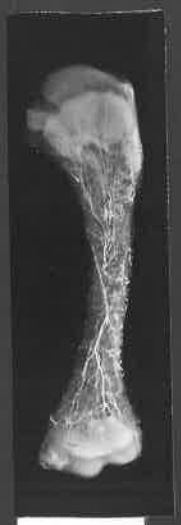


Fig.28

Fig.29a

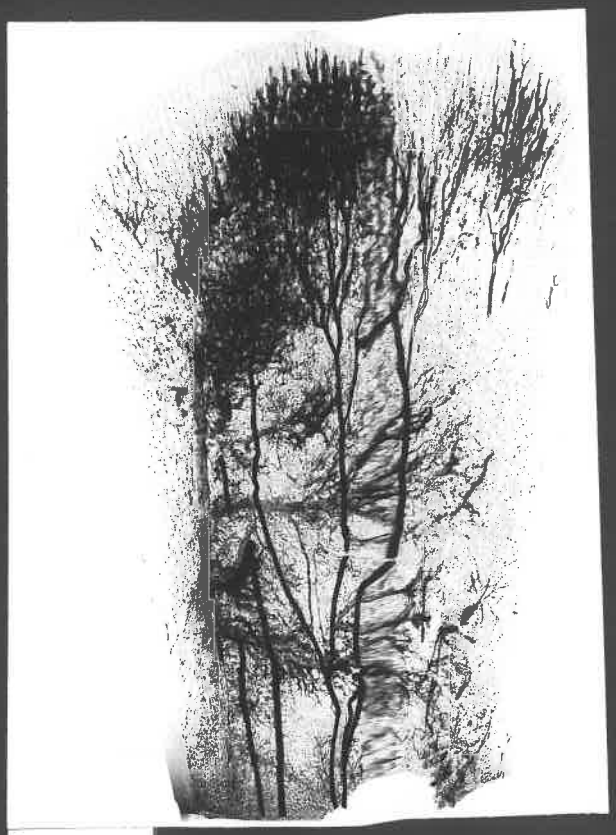


Fig.29b

running towards the site of the future foramen pneumaticum where it branches into small periosteal vessels and may give off a metaphyseal vessel. However, it does not penetrate the future pneumatic canal prior to pneumatisation.

2. Changes in vascular pattern during pneumatisation.

In the bones examined, the first sign of commencement of the pneumatisation process was the appearance, by 14 days, of a depression in the area of the future pneumatic foramen. By 21 days, the first evidence of excavation of the proximal portion of the pneumatic canal was seen, and by 28 days, the entire canal had become pneumatic, together with a small area of the shaft at the lower end of the canal (Fig. 30). It was notable, however, that there was considerable variation in the time of onset of this process. Two bones at 35 days showed full pneumatisation of the pneumatic canal with the proximal half of the shaft also being air-filled, while two others at the same age showed no more than a shallow depression at the site of the pneumatic fossa.

The most striking features of the vascular pattern in the immediate vicinity of the expanding air space are the disappearance of the normal sinusoidal pattern of the bone marrow; the appearance of an intermediate stage involving the formation of a subepithelial plexus of vessels of considerable size and number; and the final transition to the pattern of a normal capillary plexus. This remarkable alteration is almost certainly related to the mechanism of pneumatisation, and is associated

with the metabolic disappearance of fat just deep to the advancing air space. The fat cells of the normal marrow (Fig. 31) decrease in size as the fat is mobilised (Fig. 32) and transform into small cells with dense cytoplasm, which, together with the mesenchymal or stromal cells of the marrow in this area, constitute the so-called subepithelial connective tissue zone (Fig. 33).

The sinusoids present in this zone of transformation dilate markedly and become surrounded by the condensation of cells (Fig. 34). The vascular pattern immediately beneath the advancing air space is therefore that of a subepithelial plexus of rather large vessels (Fig. 35). The change is associated with vigorous osteoclastic activity in the area (Fig. 36).

As pneumatisation advances and the lining epithelium comes into closer proximity with the endosteum, the intervening zone of mesenchymal connective tissue becomes progressively reduced in width (Fig 37). The main vessels and their branches are of course located in this layer and may often be seen bulging into the air space to some degree. In addition, however, it is notable that the dilated vessels of the advancing zone of subepithelial tissue finally become replaced by a capillary network (see later).

The entire series of vascular changes is the result of modifications involving the pre-existing intraosseous tissues and vessels. With the exception of the artery of the pneumatic canal, the new vessels do not enter the bone from without, but belong to the bone as does the subepithelial

Fig. 30. 28 day old humerus, showing pneumatisation of the pneumatic canal and a small area of the shaft. The sinusoidal marrow pattern has disappeared in the region of the air space. Spaltenholz preparation x 16.

Fig. 31. Normal fatty marrow of chick humerus prior to pneumatisation. The sinusoids are filled with perfusion materal. H & E x 500.

Fig. 32. Just deep to the advancing air space, the fat cells decrease in size and become transformed into small cells with dense cytoplasm. The sinusoids dilate markedly. H & E x 200.

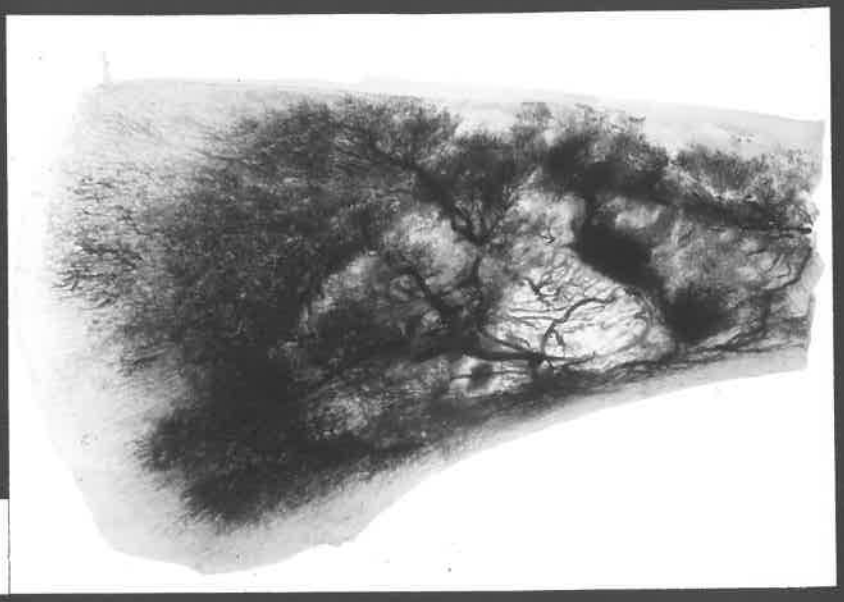


Fig.30

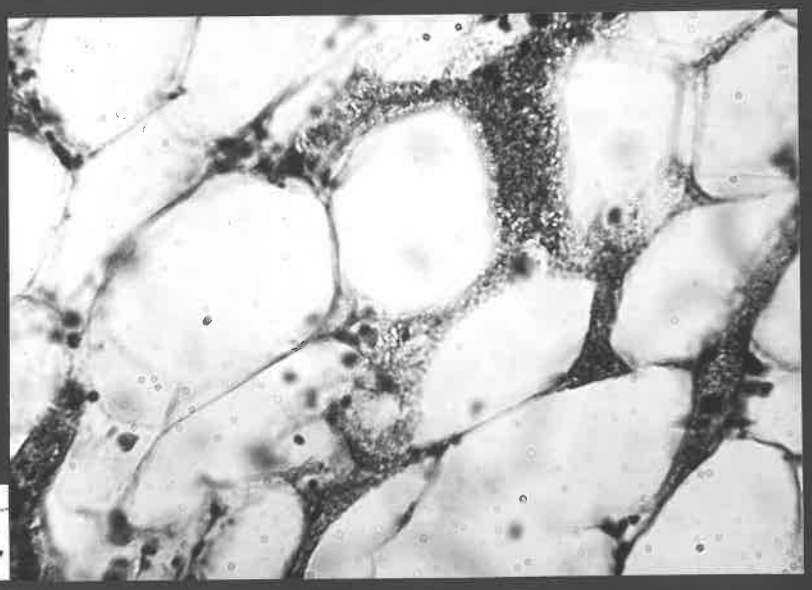


Fig.31

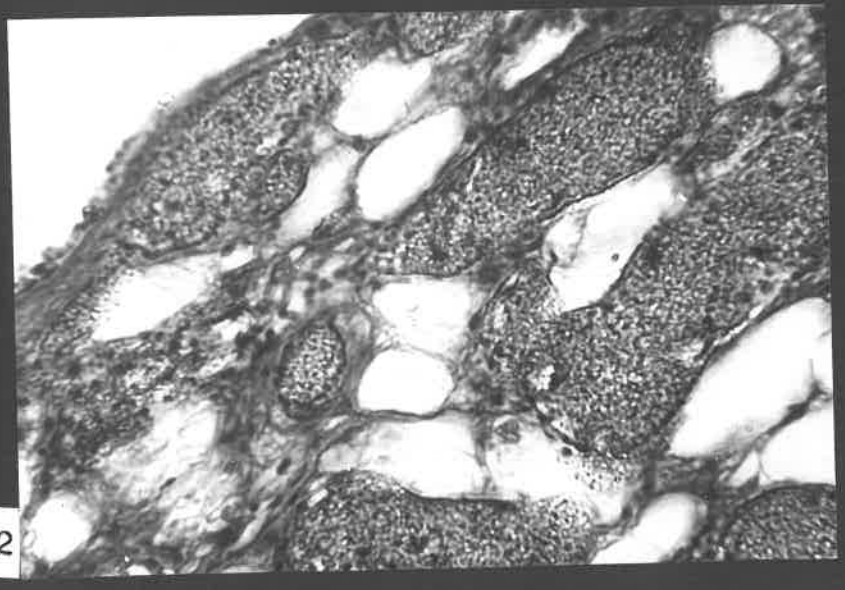


Fig.32

Fig. 33. Subepithelial mesenchymal connective tissue zone with perfused vessels. Partially pneumatised humerus. H & E x 100.

Fig. 34. H.P. view of perfused, dilated sinusoid surrounded, at one end, by a condensation of cells. H & E x 500.

Fig. 35. Subepithelial vascular plexus beneath the advancing air space (H.P. of Fig. 30). Spalteholz preparation x 36.

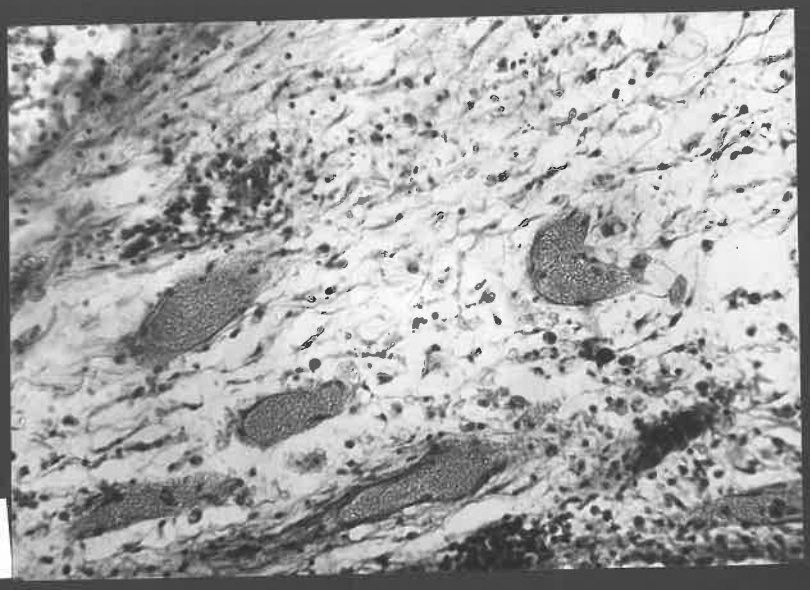


Fig.33

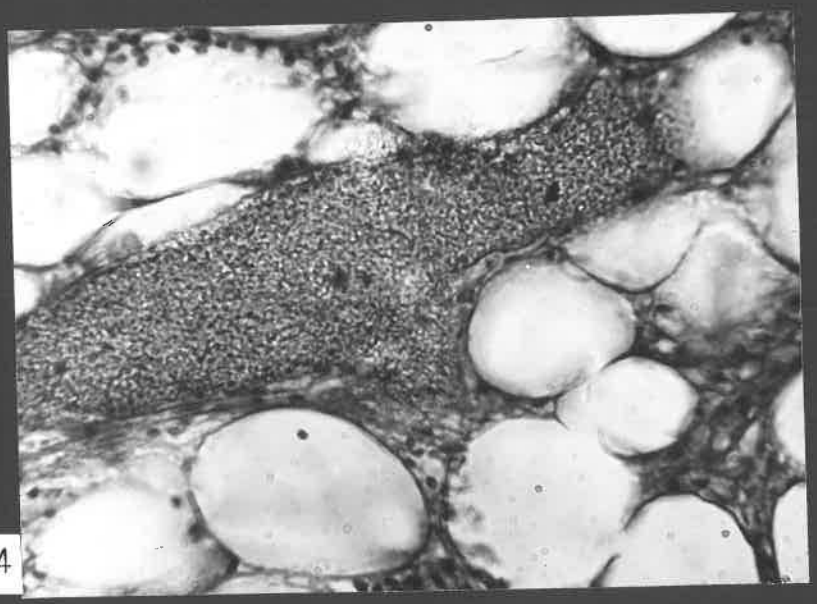


Fig.34

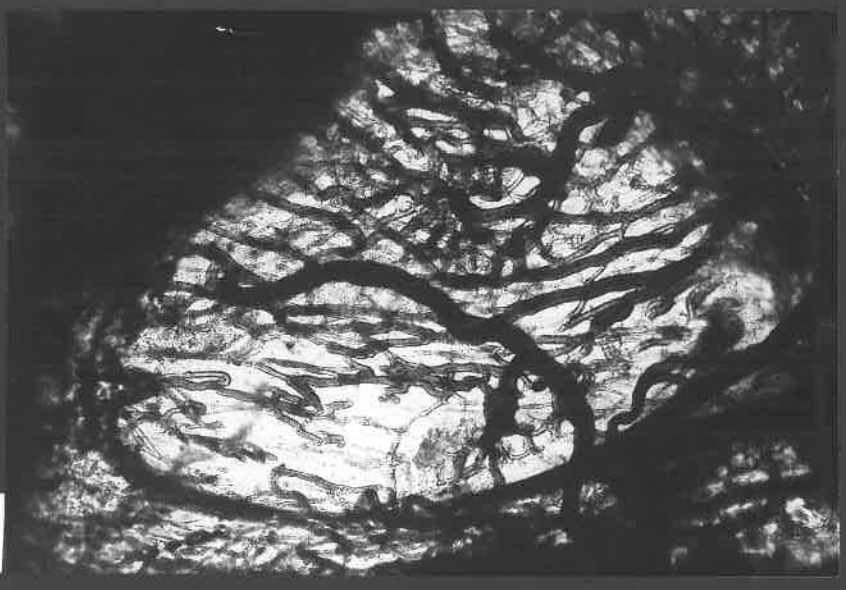


Fig.35

Fig. 36. Osteoclastic activity in association with the vascular changes of pneumatisation. H & E x 200.

Fig. 37. Fully pneumatised control humerus, showing the lining of the air space with its narrow zone of vascular mesenchymal tissue. H & E x 700.

Fig. 38. Partially pneumatised humerus. The intraosseous vessels are displaced peripherally as the air space increases in size. Spalteholz preparation x 2.

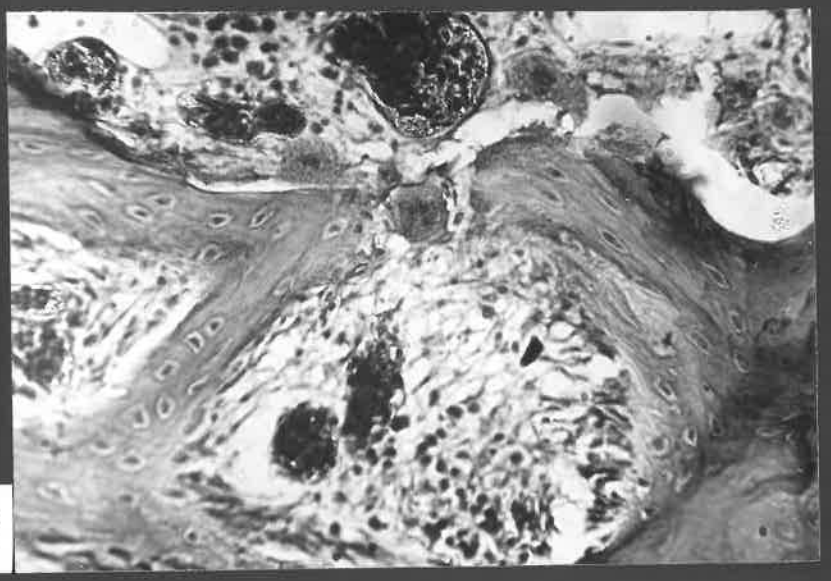


Fig.36

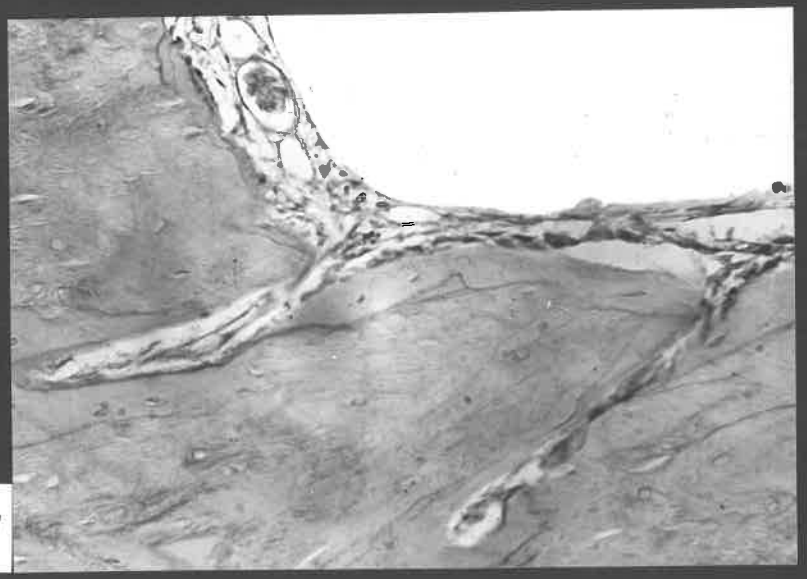


Fig.37

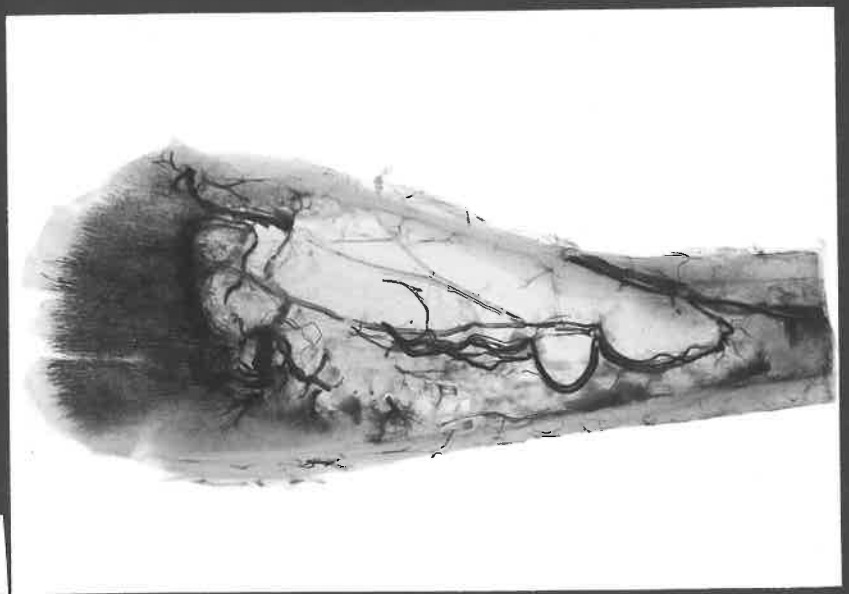


Fig.38

connective tissue zone in which they lie.

The artery of the pneumatic canal does not exist in the bone prior to pneumatisation. It is first seen giving rise to a number of periosteal branches in the region of the future foramen pneumaticum. A little later, the foramen pneumaticum is excavated, it sends one or more branches through the cortex to the metaphyseal surface of the growth cartilage, and others to the cartilaginous epiphysis. It does not send branches down the pneumatic canal into the air space region until some time after pneumatisation has commenced.

Its final distribution, best observed in fully pneumatised specimens, is restricted to the upper third of the bone (see later), so that new vascular patterns developing below this level certainly cannot be the result of vessels introduced "from without".

3. Vascular pattern following pneumatisation.

Following pneumatisation, the most striking features of the pattern of the intraosseous vessels of the humerus are the gross displacement of vessels from their former course; the prominence of the artery of the pneumatic canal; an apparently great reduction in vascularity of the interior of the bone, as compared with a marrow containing bone when viewed radiologically following perfusion; and the absence of a simusoidal pattern in all except the remaining areas of marrow.

a. The arterial supply.

The displacement of the vessels is a function of the disappearance

of marrow from the shaft. The vessels are shifted towards the endosteal surface of the cortex (Fig. 38), and may run along, or sometimes through, the bony trabeculae which weave across the air space. They lie in the subepithelial tissue which remains between the endosteum and the air space lining.

The ascending division of the nutrient artery is always displaced laterally, with a tendency to run anterolaterally or posterolaterally on occasions. However, after arising from the main stem of the nutrient artery and before beginning its lateral ascent, the vessel first winds horizontally, or occasionally obliquely, around the inner surface of the cortex, lying either anteriorly or posteriorly. Rarely, it may pass directly across the shaft along trabeculae. The direction of shift of this portion of the ascending division determines the shift of the descending division of the nutrient artery. When the horizontally running portion of the ascending division is displaced anteriorly, the descending division tends to run downwards in an anterior, anteromedial or medial position. When it is displaced posteriorly, or runs directly across the air space, the descending division runs downwards either posteriorly or posteromedially. The descending division was not seen to run downwards in a lateral position in any specimen examined.

This general displacement pattern (Fig. 39) is modified by deviation of vessels along, over, or through trabeculae so that any given vessel may appear to run a somewhat tortuous and devious course when observed

radiologically or in Spalteholz preparations. Nevertheless, the normal pattern of splitting of the divisions of the nutrient artery into their main branches and sub-branches can often still be made out despite the distortion, especially at the lower end of the bone.

In well perfused specimens it is possible to trace the terminal ramifications of the nutrient system as far as the growing end of the bone (Fig. 40). However, in the majority of bones it appears that the metaphyseal vessels, including the artery of the pneumatic canal, and, at a later stage, the arcuate vessels originally of the cartilaginous ends, may very greatly supplement, and possibly largely take over, the supply of the bone ends.

This is especially noticeable with regard to the artery of the pneumatic canal (Fig. 41), which, after approaching the pneumatic fossa and giving off a twig which passes across the apex of origin of the medial head of the triceps towards the medial tubercle of the humerus, describes a loop directed proximally before passing down the length of the pneumatic canal into the general air space. The anatomy of its branches is variable. In general it gives rise to the following intraosseous vessels:-

(i) proximally running vessels arising from the summit of the loop of the parent vessel and passing towards the growing end of the bone. They supply branches to the medial and central areas of the metaphyseal aspect of the growing end.

(ii) minute twigs, either from the parent vessel or from one or

Fig. 39. Fully pneumatised humerus (P.A. and lateral). Arterial perfusion showing general pattern of arterial displacement. Radiograph.

Fig. 40. Humerus, pneumatisation almost complete (P.A. and lateral). Arterial perfusion. The ascending and descending divisions of the nutrient artery can both be traced to the growing ends of the bone. The descending division is displaced anteriorly rather than posteriorly as in Fig. 39.
Radiograph.

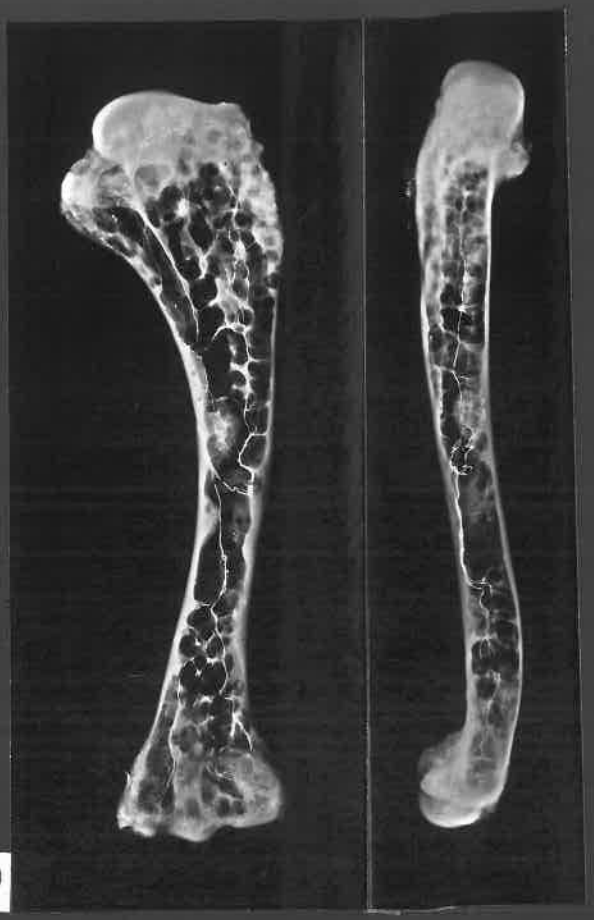


Fig.39

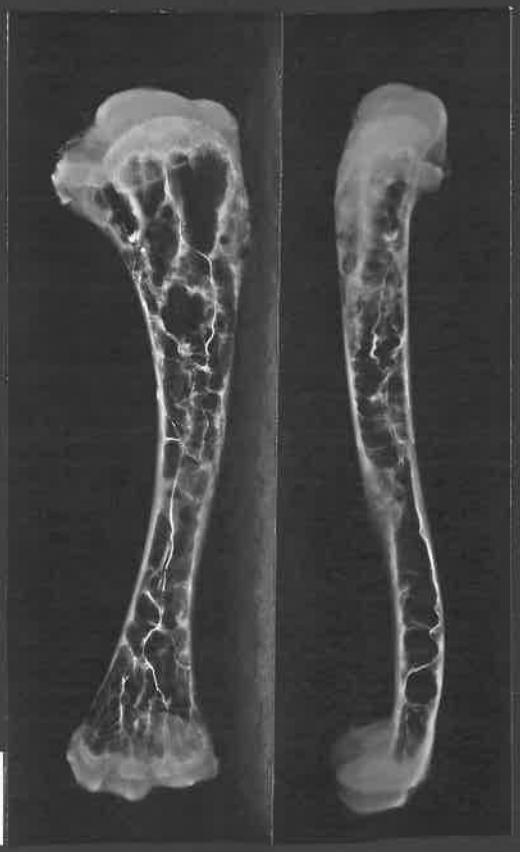


Fig.40

more of its branches, which form a circular anastomosis around the rim of the foramen pneumaticum.

(iii) descending branches which pass down the pneumatic canal with the parent vessel for a variable distance before passing through apertures in the side walls of the canal. Many of these tend to curve proximally again after they have passed out of the canal. They wind tortuously along trabeculae and supply the medial and central areas of the proximal third of the air space.

The overall distribution of this vessel is strictly limited to the proximal third of the bone.

b. The capillary network.

As previously mentioned, on radiological examination of perfused specimens there appears to be a general reduction in vascularity following pneumatisation. Part of the reason for this is undoubtedly the development of a vast subepithelial capillary network replacing the sinusoidal pattern in all areas except where a little marrow remains. Being smaller than sinusoids, the capillaries do not permit the micropaque particles to pass and therefore cannot be seen radiologically. However, Berlin blue can be made to permeate the system which can then be examined in Spalteholz preparations (Fig. 42).

c. The venous system.

By using the venous perfusion technique, the veins of the fully pneumatised humerus can be examined separately from the arterial supply. As a result of this method, it was established that the pattern of

Fig. 41. Fully pneumatised humerus, proximal half. Arterial perfusion showing the course and distribution of the artery of the pneumatic canal. Spalteholz x 2.

Fig. 42. Subepithelial capillary plexus of fully pneumatised humerus. Microdissection of Spalteholz preparation x 25.

Fig. 43. Fully pneumatised humerus. Venous perfusion showing general pattern of venous drainage. Radiograph.

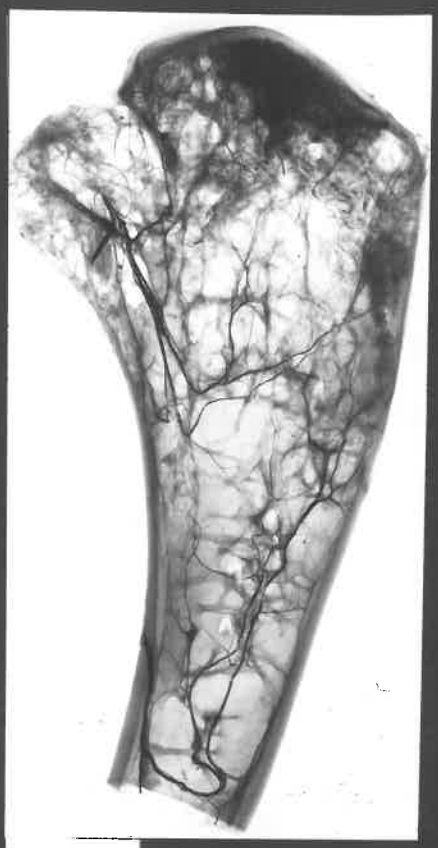


Fig.41

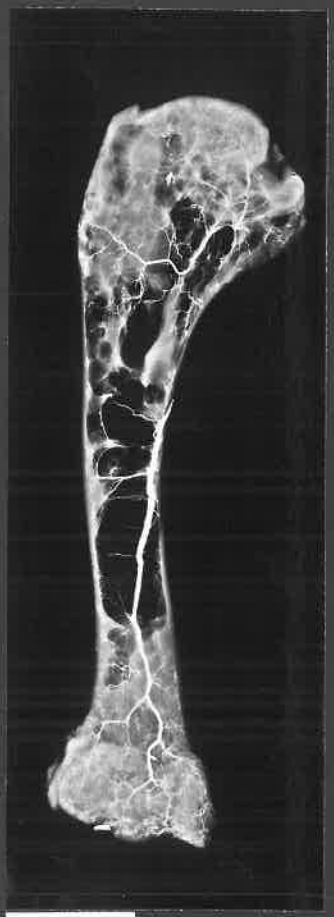


Fig.43

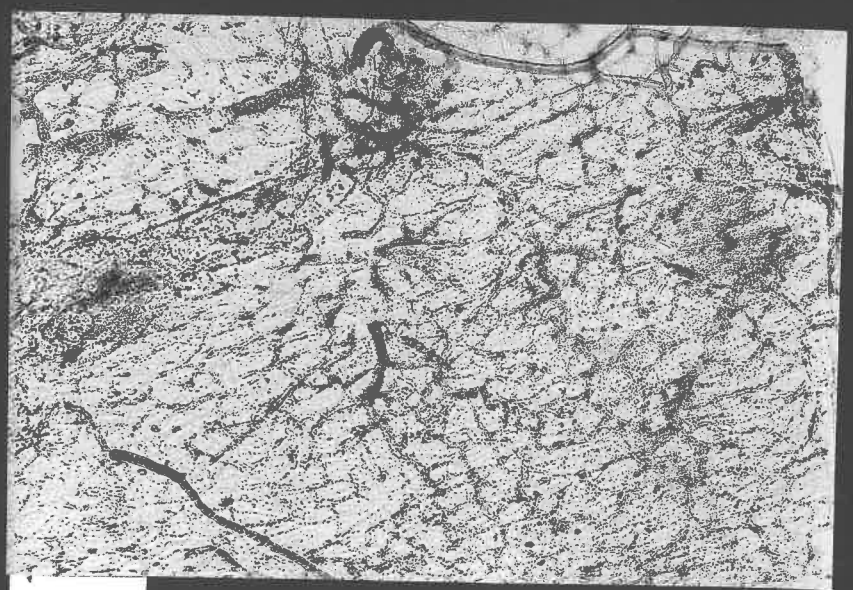


Fig.42

displacement of the veins corresponds with the pattern of arterial shift (Fig. 43).

However, the medullary vein becomes largely confined to the lower two thirds of the humerus. Commencing at the distal end of the bone by the union of several small tributaries, it proceeds proximally to its oblique exit through the nutrient foramen. Numerous small tributaries enter it more or less at right angles during its course.

The venous drainage of the proximal third of the bone tends to be taken over by the vein of the pneumatic canal. The pattern of the tributaries of this vein is considerably modified by the pneumatisation process. The arrangement which is seen prior to pneumatisation becomes progressively altered until the general configuration finally resembles the branching pattern of the corresponding artery.

Many additional veins are found at each end of the bone after the completion of growth. These represent vessels which originally drain the cartilaginous epiphyses prior to closure of the growth plate. They therefore correspond to the arcuate vessels previously described.

In well-perfused specimens, numerous small vessels may be seen forming an extensive web which links up these major drainage areas.

3. The fate of the air cells of the humerus of Gallus domesticus after surgical blockage of the foramen pneumaticum.

Introduction.

The foramen pneumaticum was blocked in the humeri of 44 cockerels,

the first group of 24 being fully pneumatised, the second group of 20 being partially pneumatised.

Assessment of the stage of pneumatisation was made radiologically and only those birds in which development of the air space appeared to be complete were accepted for the first group. For the second group it was felt that the air space needed to be sufficiently well developed for it not to be totally filled by an inserted muscle graft, but also not so far advanced that pneumatisation had reached virtual completion. X-ray studies of developing birds were therefore repeated until a suitable stage of pneumatisation had been reached. Exact measurements of the extent of the air space were not possible as there was rarely a sharp radiological demarcation between the air space and the marrow. In addition, there was a considerable age variation in relation to the stage reached (see Table I). When approximately $\frac{1}{3}$ to $\frac{2}{3}$ of the total bony shaft length was pneumatised, the situation was considered satisfactory.

The two groups were each subdivided into two subgroups. In one subgroup of each main group, the foramen pneumaticum was blocked with methyl methacrylate. In the other subgroup of each main group the foramen pneumaticum was obliterated with a pedicled muscle graft. Each of the four subgroups was then arranged so that serial sacrifice could be carried out weekly for four weeks, and monthly up to a period varying from five to eight months (see Table II).

The exact ages of the animals were known only in the partially

TABLE I.

Age variation in relation to the stage of Pneumatisation.

Approximate stage of Pneumatisation	Age, in days, at X-ray.								
	33	47	50	54	56	63	68	82	110
Nil	+	+	+						
¼	+++ +++ +	+	+	+	+				
⅓	+++	+++ ++	+	++	+	+			
½		+++ +++	+++	+++ +++		+++	+	+	
⅔		+++ +	+	+			+		
¾		+++	+	+	+	+	+		+
Full								+++	++

(+ represents one bird).

TABLE II.

Plan of Serial Sacrifice.

1st Group - Fully pneumatised series. (18 birds).

Methyl Methacrylate		Muscle Graft	
Number of bird	Survival time.	Number of bird	Survival time.
C.B. 21	1 week	C.B. 24A	1 week
C.B. 23A	2 weeks	C.B. 12	2 weeks
C.B. 3	3 weeks	C.B. 13	3 weeks
C.B. 4	1 month	C.B. 14	1 month
C.B. 5	2 months	C.B. 18	2 months
C.B. 6	3 months	C.B. 19	4 months
C.B. 24	4 months	C.B. 20	"
C.B. 23	5 months	C.B. E	5 months
C.B. 9	6 months		
C.B. 10	8 months		

TABLE II.

Plan of Serial Sacrifice.

2nd Group - Partially pneumatised series (20 birds).

Methyl Methacrylate.				Muscle Graft.			
Number of bird	age at operation	Survival time	Age at Sacrifice	Number of bird	age at operation	Survival time	Age at Sacrifice
CB 3A	54 days	1 week	61 days	CB 13A	57 days	1 week	64 days
CB 22	50 days	2 weeks	64 days	CB 25	54 days	2 weeks	68 days
CB 32	50 days	3 weeks	71 days	CB 15	54 days	3 weeks	75 days
CB 35	47 days	1 month	75 days	CB 4A	54 days	1 month	82 days
CB 25	51 days	2 months	107 days	CB 40	54 days	2 months	110 days
CB D	56 days	3 months	140 days	CB 36	51 days	3 months	135 days
CB 27	56 days	4 months	168 days	CB C	70 days	4 months	182 days
CB A	55 days	5 months	195 days	CB B	70 days	5 months	210 days
CB 30	51 days	7 months	247 days	CB 31	56 days	7 months	252 days
CB 29	55 days	8 months	279 days	CB 34	55 days	8 months	279 days

pneumatised series, and in five of the fully pneumatised series. However, this knowledge was not essential to the experiment or its interpretation.

Six of the birds of the first main group were used to establish the surgical technique and excluded from Table II.

A selection of 18 experimented humeri were perfused following sacrifice and were examined radiographically after decalcification. The technique of vascular perfusion differed in no way from that previously described, 11 being injected arterially, and 7 being injected venously. The bones were selected f rom both main series and were chosen in order to demonstrate changes in the vascular pattern at periods varying from one week to eight months after operation (Table III).

Except for two bones which were taken through the Spalteholz process prior to histological study in order to examine further vascular details, the remainder on which any experimental procedure had been carried out were studied macroscopically and then submitted for histological examination.

Observations.

1. The external appearance of the operated bones.

All surgical procedures were carried out with sterile precautions, and each bird was given an injection of penicillin intramuscularly at the completion of the operation. With these measures alone, no evidence of infection was seen. All wounds healed rapidly by first intention, and at sacrifice the area of operation appeared scarred but without any evidence of local infection. The axillary air sac looked normal and

TABLE III.

Vascular studies on experimented humeri.

	Number of Bird	Post-operative Survival time	Type of Injection	Series of Experiment.
1.	C.B. 21	1 week	Arterial	1st Group-Sevriton
	C.B. 23A	2 weeks	"	"
	C.B. 18	2 months	"	1st Group-Musc.Graft
	C.B. 20	4 months	"	"
5.	C.B. 19	"	Venous	"
	C.B. 24	"	"	1st Group-Sevriton
	C.B. 27	"	Arterial	2nd Group-Sevriton
	C.B. C	"	"	2nd Group-Musc.Graft
	C.B. B	5 months	"	"
10.	C.B. 12A	"	"	1st Group-Sevriton
	C.B. 23	"	Venous	"
	C.B. E(R)	"	"	"
	C.B. E(L)	"	"	1st Group-Musc.Graft
	C.B. 9	6 months	Arterial	1st Group-Sevriton
15.	C.B. 30	7 months	"	2nd Group-Sevriton
	C.B. 31	"	Venous	2nd Group-Musc.Graft
	C.B. 29	8 months	"	2nd Group-Sevriton
18.	C.B. 10	"	Arterial	1st Group-Musc.Graft

"Sevriton simplified" is the trade name for the preparation of methyl methacrylate used.

healthy in all cases.

All operated bones felt markedly more heavy than the corresponding control bones. The exact increase in weight could not be measured as a routine procedure because of the perfusion studies and the immersion in fixative prior to periosteal stripping. However, in the case of one bird sacrificed after two months (C.B.5), both control and experiment bones were meticulously stripped of all soft tissues and periosteum, and weighed immediately after sacrifice. The weight increase on the operated side was 5.7 Grams. The weight of the methyl methacrylate block was only 0.05 Grams.

Except in five birds, where the controls showed evidence of a previous fracture and where the resultant deformity prevented any valid comparison, the operated bones showed no obvious variation in length as compared with the corresponding controls. Measurements of total bone length were made from longitudinal histological sections and, within the limits of the method and the variation of plane of section, the results obtained supported this impression.

Another outstanding feature was the alteration in general colour and translucency of the operated bones. All normal, pneumatised, uninjected control bones were of creamy-white colour, and, when held to the light, appeared somewhat translucent, except for the darker shadows of the trabeculae within the air space. The operated bones, on the other hand, especially the earlier ones of the series, were distinctly reddish-brown in colour, due to considerable vascular congestion. On viewing the

bones against light, the earlier ones of the series appeared considerably less translucent than the controls, while the later bones were opaque to light. In some the appearance was a patchy combination of these features.

2. The interior of the operated bones.

For reasons given in the section on methods the bones could not be routinely cut open until after fixation and decalcification. In many cases, therefore, the naked-eye appearance of the fresh air space lining and of the contents of the pneumatic system could not be examined. However, several unperfused, experimental bones were specifically opened in the fresh state. At four weeks, and again at two months, the lining membrane appeared shiny, swollen and oedematous. The air space contained a thin, serous fluid which varied from a clear, yellowish colour to an opalescent, slightly pinkish colour. The pink colour was probably the result of admixture with a little blood while opening the bone. At six months, the air space was seen to contain a considerable amount of new tissue interspersed with small cystic spaces containing fluid similar to that previously seen. At no stage was any thick, mucoid fluid extracted from the bones.

Specimens of the fluid were examined microscopically and under polarised light. All contained occasional red cells, possibly the result of slight trauma to the lining tissues while obtaining the specimens, and a considerable amount of protein material. The most interesting feature, however, was the presence of doubly-refractile, elongated, flat crystalline plates (or needles if viewed edge-on). These showed coloured rings,

first bright carmine-red and then violet, when acted upon by a mixture of five parts conc. sulphuric acid and one part water (Hammersten 1904). They were undoubtedly crystals of cholesterol. Droplets of fat were also present. It must be noted that neither the cholesterol crystals nor the fat droplets were present in any fluid taken from bones prior to 4 weeks after operation. They were present, however, in all specimens examined from fully pneumatised bones after 5-6 weeks of obstruction of the foramen pneumaticum.

Facilities were not available for large-scale biochemical testing of fluid samples. However, three specimens of 4 weeks, 5½ weeks, and 7 weeks after blockage, were tested for the DNA content and total protein after spinning for 5 minutes at 6,000g. No DNA was found in the clear supernatant fluid from any specimen when tested according to the method of Burton (1955). The protein concentrations were 23.7 mgm/ml, 35.2mgm/ml, and 48.2mgm/ml. in the 4 week, 5½ week, and 7 week specimens respectively, as assessed by the Biuret method (Gornall, Bardawill, David 1949). Two random serum protein values were 44.6 mgm/ml. and 37.8 mgm/ml. Obviously such a limited number of results does not permit any definite conclusion. However, they suggest that the protein level rises as the duration of obstruction increases.

3. The block used.

In all the cases included in Table II, the block used was still effective at the time of sacrifice of the animals.

a) Methyl methacrylate ("Sevriton") block.

Generally this was firmly adherent to the margins of the pneumatic fossa and was overlain by a varying thickness of fibrous tissue. The fibrous reaction was mainly the result of the surgical manoeuvres required to expose the foramen pneumaticum. Sections of the tissue showed degeneration and fibrous replacement of muscle fibres in the area of the surgical approach.

The foreign body reaction to methyl methacrylate was minimal and quite localised in all cases, being best observed in sections showing the deep aspect of the block. The reaction was restricted to the formation of an encapsulating layer of fibrous tissue of varying thickness which separated the block from the underlying bony floor of the pneumatic fossa. No giant cells were observed at any stage.

In cases sacrificed soon after the block had been inserted, considerable osteoblastic activity was noted on the floor of the pneumatic fossa just deep to the fibrous capsule. It is probable that this reaction was the result of surgical trauma to the bony floor of the pneumatic fossa while preparing it for the introduction of the block rather than a reaction to the methyl methacrylate itself.

In any event, all tissue changes due to the surgical exposure or the presence of methyl methacrylate in the pneumatic fossa were strictly localised to the fossa and the overlying tissues. There was no extension of the reaction into the closed-off air space.

In two cases not included in Table II, the methyl methacrylate block

had failed to provide a permanent obliteration. It appeared to have been displaced distally by muscle adherence and pull. In these cases, pneumatisation had proceeded to completion through a semilunar opening just proximal to the block. The air space and its lining appeared normal.

b) The muscle graft.

The surgical interference in the muscle graft operation was greater than that in the "Sevriton" block procedure. Consequently, in all cases the overlying mass of fibrous tissue was greater.

The muscle graft itself was carefully fashioned so that it would not be pulled out of position by muscular action or joint movement, and so that its blood supply was preserved as far as possible. In addition, a wide opening was made into the interior of the proximal third of the shaft so that the graft might be rotated into position with minimal kinking or trauma. Radiographs (Fig. 44) and histological studies of injected grafts confirmed that the blood supply had been maintained, but despite this, all grafts underwent a progressive degeneration of muscle fibres and conversion into fibrous tissue (Figs. 45,46). This change was seen at the periphery of the graft by the first post-operative week, and proceeded until normal muscle fibres remained only along the plane of the operative opening through the bone cortex.

Progressive shrinkage of the fibrous mass in the direction of its pedicle was observed by the second or third post-operative week and usually continued until all that remained of the original graft was a

Fig. 44. Humerus, 5 months after muscle graft operation. Arterial perfusion. Blood vessels are present in the graft itself. Radiograph.

Fig. 45. Degenerative changes in muscle graft (T.S.) H & E x 100.

Fig. 46. Degenerative changes in muscle graft. (L.S.) H & E x 200.

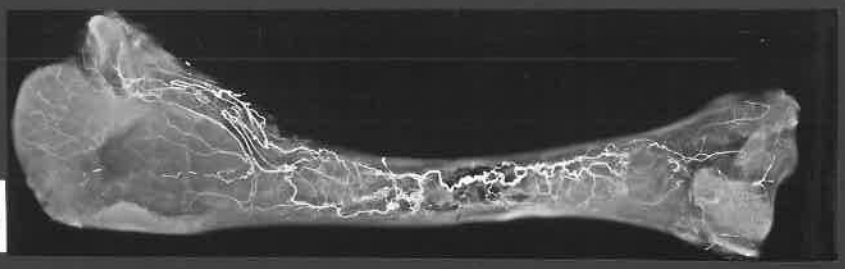


Fig.44

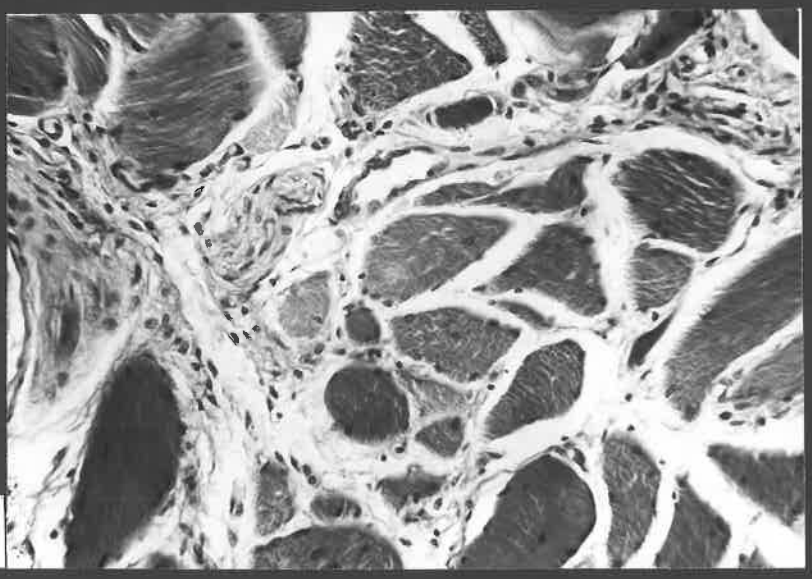
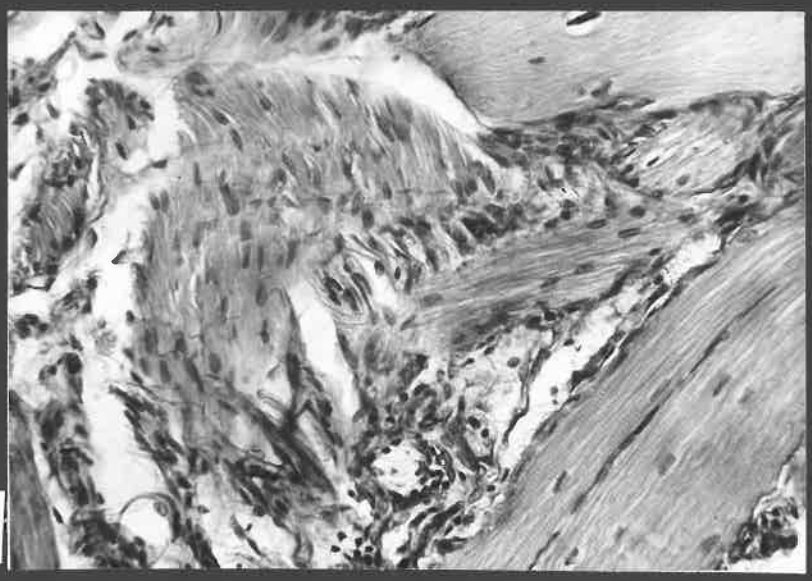


Fig.45



rig.46

musculo-fibrous mass filling the operative bony deficiency in the plane of the opening (Figs. 47-9). The mass of tissue which had previously been inserted to fill the interior of the upper $\frac{1}{4}$ - $\frac{1}{3}$ of the humerus had disappeared.

This change was seen in some cases by the end of the first post-operative month, but it was notable that there was considerable variation in the speed and degree of muscle degeneration, fibrous replacement, and shrinkage. In those cases where pneumatisation had proceeded to only a limited degree and where the inserted graft had totally filled the air space, the muscle fibre degeneration proceeded as before, but the original small air space was filled with a mass of fibrous tissue.

Failure of permanent obliteration by the muscle graft method was more frequent than by the methyl methacrylate method, and occurred in 6 bones. The failure was discovered at periods varying from two to four months post-operatively, and the bones were excluded from the main series. In these cases, however, pneumatisation had proceeded to completion. The pathway of re-ventilation was at the proximal end of the operative bony deficiency in all cases. It was probable that curettage of the air sac lining had been incomplete in this area, and that the remnant of lining tissue covering the bony rim of the foramen pneumaticum had prevented the formation of a fibrous tissue seal between the graft and the bone. Atrophy and shrinkage of the graft may have been a subsidiary factor.

Unfortunately there was no way of assessing how long the muscle graft block had been effective prior to the re-establishment of a pathway

Fig. 47. Proximal half of partially pneumatised humerus with inserted muscle graft. Degenerative changes have commenced in the graft. H & E x 4.

Fig. 48. Proximal half of pneumatised humerus with inserted muscle graft. Degenerative changes have progressed and shrinkage of the graft is occurring. H & E x 5.

Fig. 49. Proximal half of pneumatised humerus. All that persists of the inserted muscle graft is a strip of muscle closing the bony deficiency in the cortex. H & E x 5.

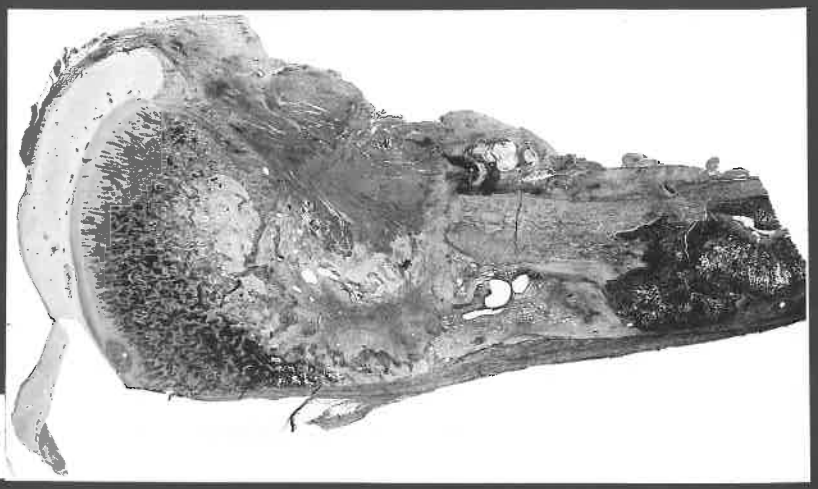


Fig.47

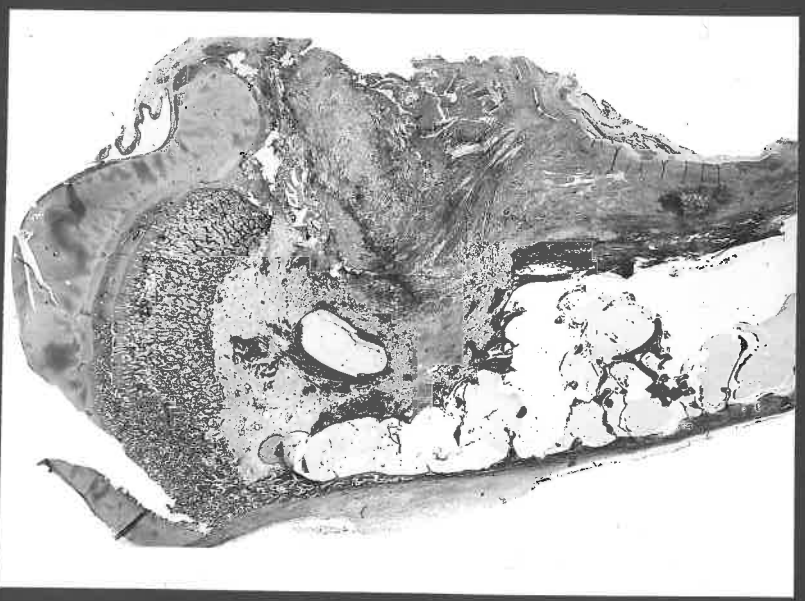


Fig.48

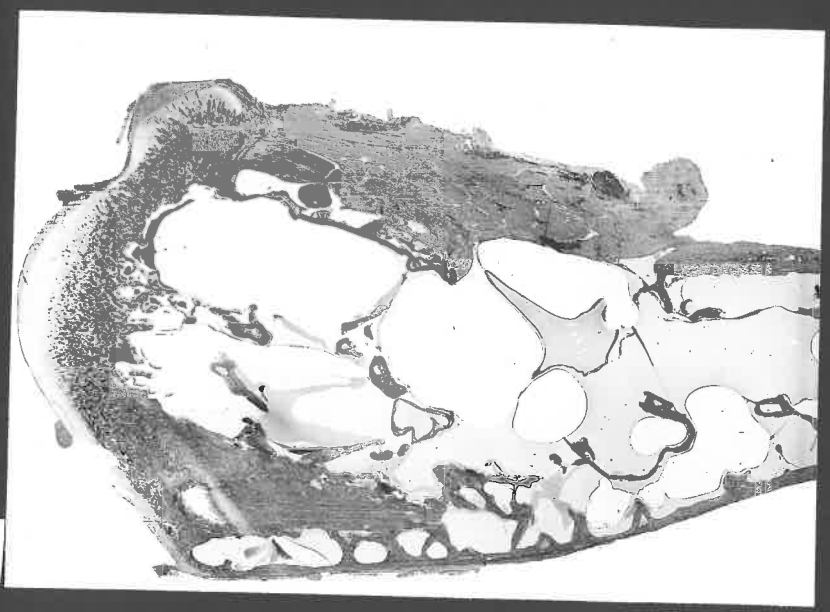


Fig.49

of ventilation. From the available material, therefore, it is not possible to assess the degree to which the pathological changes, now to be described, were reversible.

4. Histopathological changes in the pneumatic system.

Because of serial sacrifice, it was possible to observe a progressive series of histopathological events over a period extending from one week to eight months after operative blockage of the foramen pneumaticum. During this period, changes were observed in relation to the epithelium lining the air cells, the subepithelial tissues, the bone substance itself, the bone marrow and the contents and size of the pneumatic spaces. It was also noted that the various changes occurred in a definite time sequence, and that the time of onset of any given change varied according to whether the operative procedure was a muscle graft or a methyl methacrylate block, and whether the bone was partially or fully pneumatised at the time of operation.

It is, therefore, proposed to discuss the observed changes in the order of their appearance, and to point out, where significant, the influence of the particular operative procedure, and the degree of pneumatisation, on the time of onset of each change.

In no bone from which the following observations were made was there any histological evidence of infection.

(a) Changes in the lining epithelium.

The first changes were seen one week after blockage of the foramen pneumaticum. Previously flat lining cells became markedly round in

appearance. Their flat nuclei also became round. Occasionally this change gave rise to an appearance resembling a cuboidal type of epithelial arrangement, although more commonly the edges of adjacent cells no longer maintained contact with one another. An appearance resembling a collection of pebbles on a wall was then seen. (Fig. 50).

At any one time, the degree of swelling varied with individual cells and, in addition, some cells seemed to become loosened. These factors were sometimes associated with a multilayered appearance of areas of the affected lining. In general, however, the lining remained only one cell thick.

The cytoplasm of the altered lining cells soon became vacuolated, and, as the change progressed, the cells became detached and floated free in the fluid-filled air space (Fig. 51). The foaminess of their cytoplasm then increased and the nucleus of each free cell condensed to a small mass (Fig. 52). Perl's stain confirmed that there was no haemosiderin present in these cells, and an Alcian blue stain demonstrated that no mucin was present. Certainly, no goblet cells were seen. However, by staining frozen sections with "Oil Red O", it was possible to demonstrate that the foamy cells were laden with fat (Fig.53).

The first area to be affected was the lining over the advancing front of the air space in partially pneumatised bones (Fig. 54). Changes were seen here after one week of ventilatory obstruction. It should be noted that this area is the most highly vascular region in the pneumatising bone. Other areas of epithelium became affected later.

Fig. 50. The flat lining cells become markedly round in appearance following operation. Adjacent cells do not necessarily remain in contact. H & E x 200.

Fig. 51. Post-operative foamy changes in the lining cells. Many of them float off into the fluid-filled air space. H & E x 200.

Fig. 52. Foam cells lying free in the fluid-filled air space. Their nuclei are greatly condensed. H & E x 500.

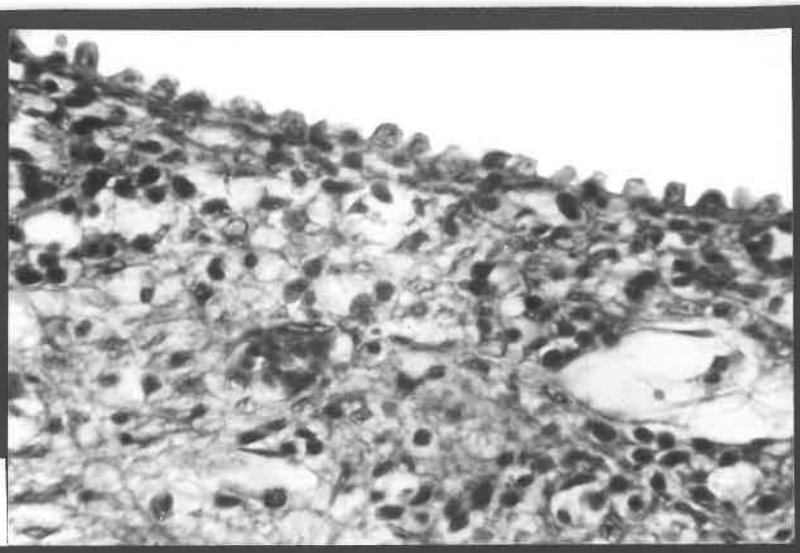


Fig.50

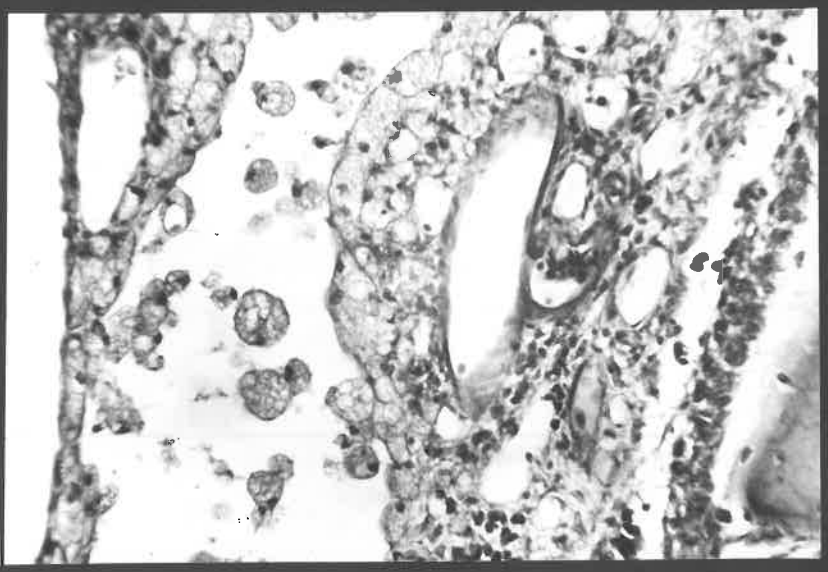


Fig.51

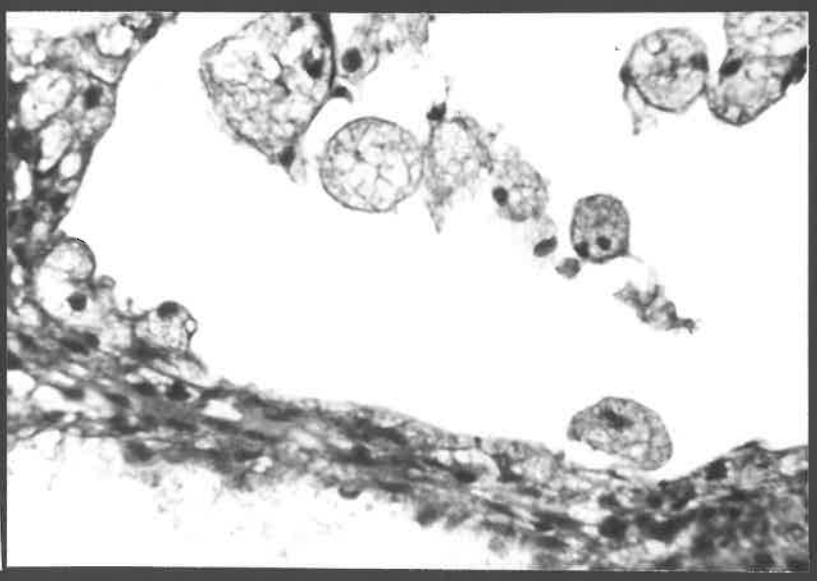


Fig.52

These areas increased in number and extent with the duration of the experiment, developed more rapidly in partially pneumatised bones than in fully pneumatic bones, and occurred more rapidly after a muscle graft than after a methyl methacrylate block.

At no stage, however, not even after eight months of ventilatory blockage, did the entire lining appear uniformly affected. In fact, one striking feature of the change was its lack of uniformity. Affected areas were often found scattered along stretches of apparently normal flat lining (Fig. 55).

No defect in the continuity of the lining epithelium was ever seen as a result of these changes, and it seemed that as the lining cells separated off, subepithelial mesenchymal cells filled the gaps from below, undergoing a transformation into flattened surface cells.

b). Changes in the subepithelial tissues.

The changes taking place in the subepithelial tissues were complex and may be divided into six phases:-

(i) initial vascular changes.

(ii) oedema of subepithelial tissues.

(iii) mesenchymal proliferation.

(iv) fatty and myeloid changes.

(v) tissue reactions to cholesterol.

(vi) new bone formation in mesenchymal tissue.

For convenience and simplicity, these will be considered separately.

Fig. 53. Frozen section stained with "Oil Red O", showing the fatty change in the rounded-up and foamy lining cells. x 200.

Fig. 54. Lining epithelium over the advancing front in a partially pneumatised bone following operation. The lining cell changes and the underlying oedema are seen. H & E x 200.

Fig. 55. Lining cells adjacent to those seen in Fig. 54. The cells remain flattened and fairly normal in appearance. H & E x 200.

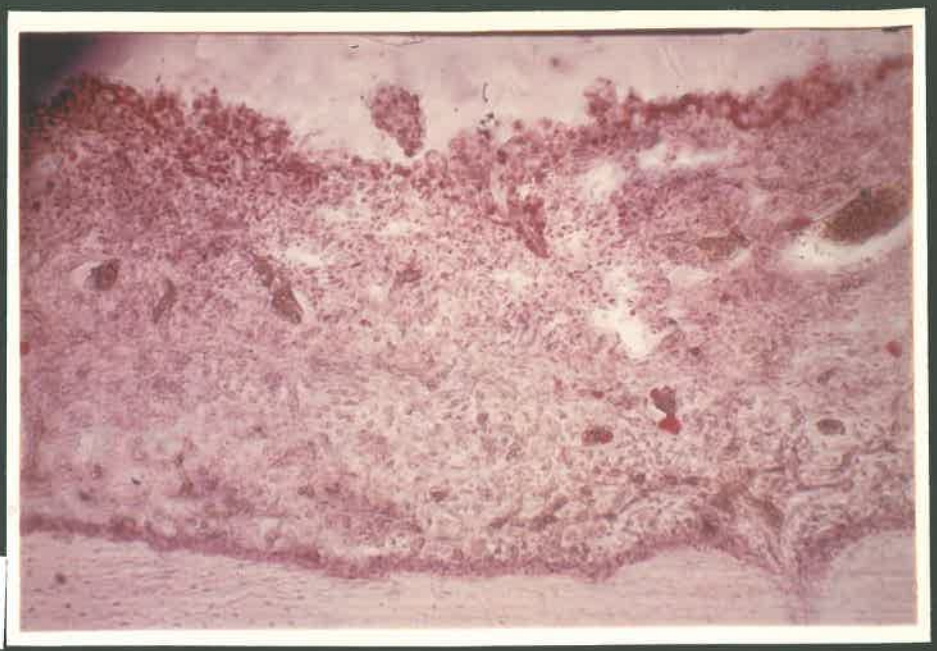


Fig.53

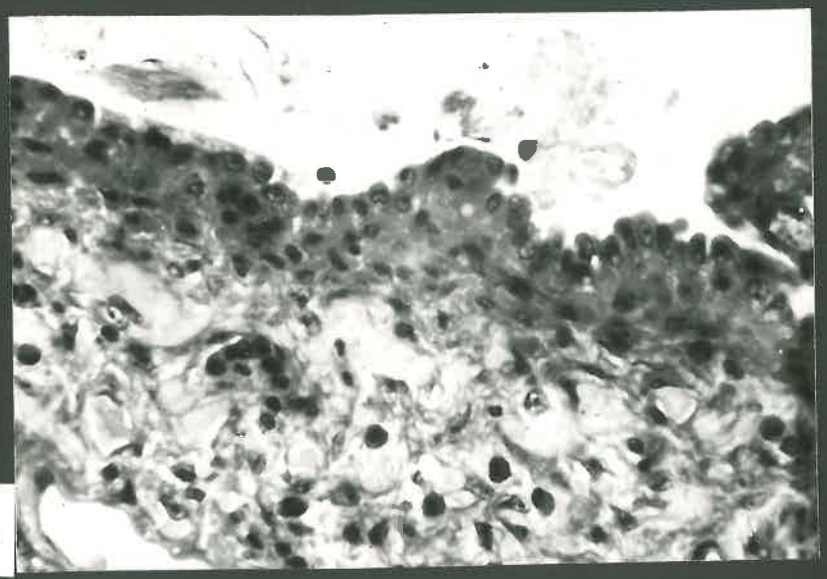


Fig.54

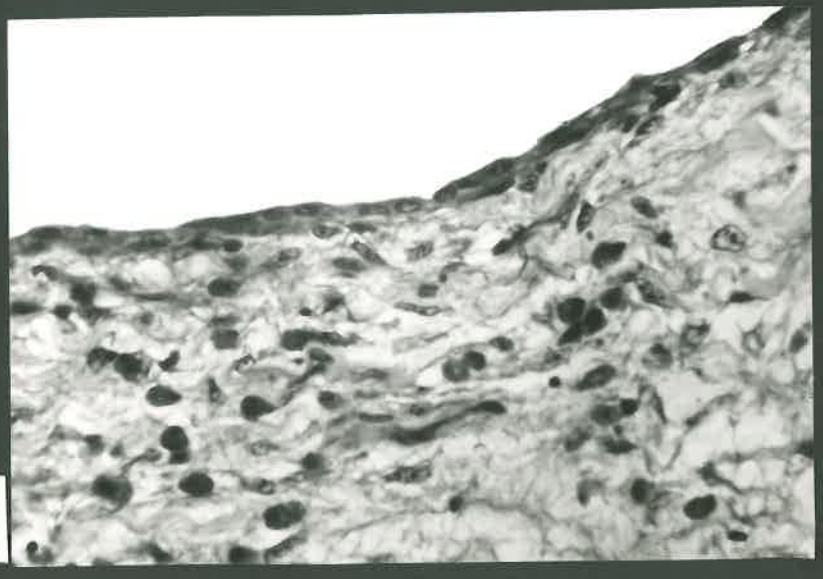


Fig.55

(i) Initial vascular changes.

As previously mentioned, the early post-operative bones appeared somewhat reddened due to congestion and dilatation of vessels. This change was observed as early as one week after obstruction of the pneumatic foramen and was studied histologically, radiologically and by the Spalteholz technique.

As seen histologically, the chief vessels affected were those of the tissue immediately beneath the lining of the air space. The existing vessels in this tissue, especially in the normally highly vascular region of the advancing front of partially pneumatised bones, became considerably dilated and congested, the most superficial ones often being seen to cause the epithelium to bulge into the air space. These vessels were well demonstrated in Spalteholz preparations (Fig.56).

The radiological examination of bones perfused one and two weeks after operation was also interesting. Compared with perfused controls, the arterial system of these early postoperative bones filled easily, a profusion of minute vessels was revealed, and it was notable that some of the perfusion material passed through the dilated capillary network to the venous side of the vascular system (Fig. 57). The degree of vascularity as assessed radiologically was much greater in the two week than in the one week bone, but strict comparison was made difficult by virtue of the impossibility of perfusing two separate bones equally.

(ii) Oedema of subepithelial tissues.

The first histological evidence of oedema in the subepithelial

tissues was observed in the region of the pneumatising front in partially aerated bones one week after obstruction of the foramen pneumaticum. The extra tissue fluid first collected in a narrow zone immediately deep to the epithelium lining the air space in this area, but as the quantity increased it spread more widely, and the whole thickness of the subepithelial tissue eventually became oedematous. Not all areas were involved to the same degree at any one time, however, and portions of extremely thin and almost normal lining were often found amongst grossly oedematous areas of subepithelial tissue.

Histologically recognisable post-operative oedematous changes became extensive much earlier in partially pneumatised bones than in fully pneumatised bones. In the partially pneumatised group, lining oedema was present one week after operation and had become extensive and generalized by 2-3 weeks. On the other hand, although there was some evidence of subepithelial oedema after 1-2 weeks in fully pneumatised bones, it did not become a marked feature in this group until at least 2-3 months after operation.

(iii) Mesenchymal proliferation.

After it had become congested and oedematous, the subepithelial connective tissue zone was next seen to increase in width by virtue of a dramatic proliferation of mesenchymal cells (Fig. 58). The change was by no means uniform in appearance, some regions of the lining being affected more than others. Areas of apparently normal lining were found adjacent to areas involved by the proliferative process.

Fig. 56. Perfused, dilated vessels in the subepithelial tissue of the humerus after blockage of the foramen pneumaticum with methyl methacrylate. Spaltehols preparation x 25.

Fig. 57. Pneumatised humerus, two weeks after "Sevriton" operation. Arterial perfusion. A host of small vessels is revealed, and the perfusion material has started to enter the venous side of the vascular system of the bone. Radiograph.

Fig. 58. Mesenchymal proliferation. Clefts are seen between adjacent polypoid masses. These become obliterated as proliferation continues. H & E x 100.

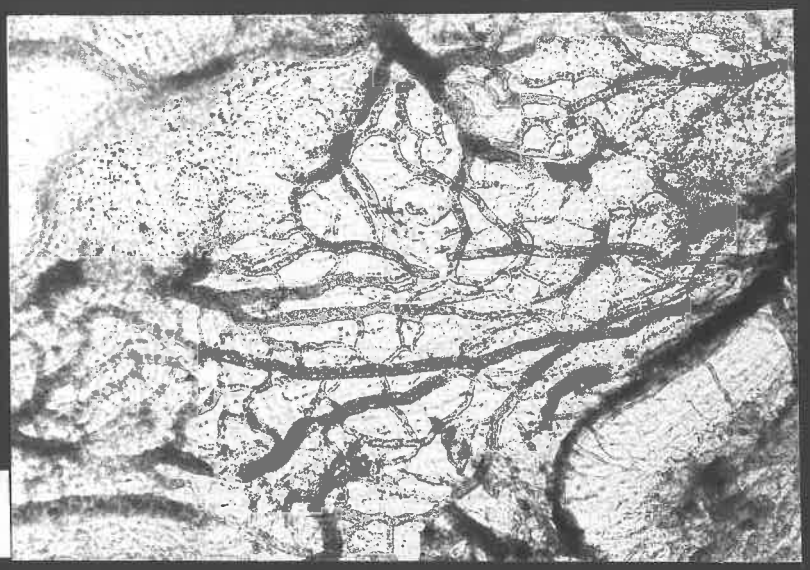


Fig.56

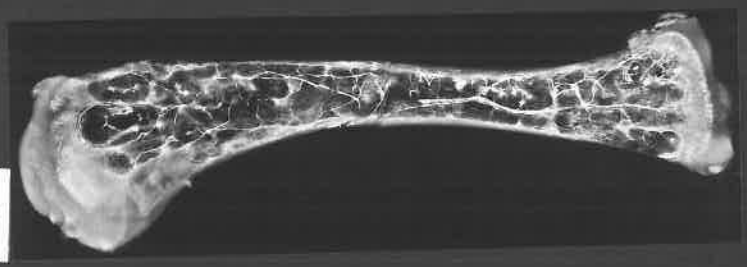


Fig.57

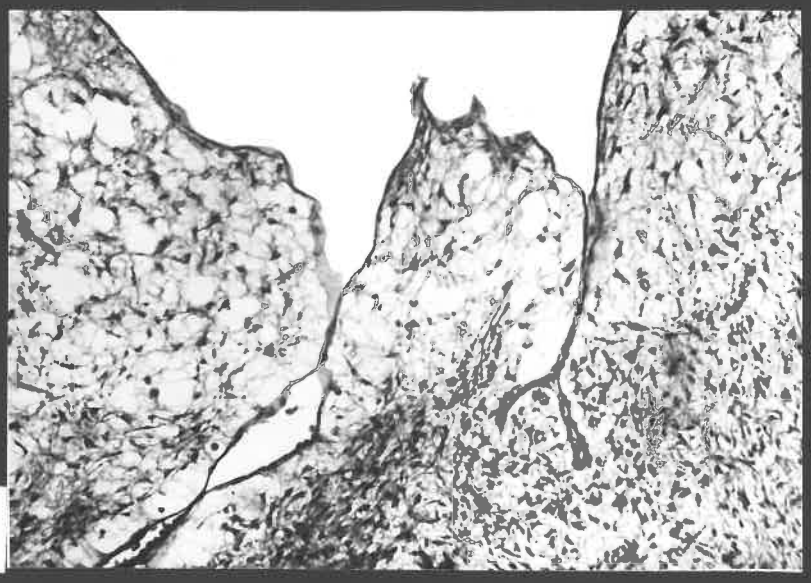


Fig.58

In addition to giving rise to a simple increase in the thickness of the subepithelial tissue, the mesenchymal tissue proliferated out into the air space itself so that bizarre patterns were found in histological sections. The newly-formed mesenchymal tissue was quite loose and consisted of cells with round, oval or elongated nuclei, and delicate, wavy, cytoplasmic arms extending out into the intercellular substance (Fig. 59). Tiny, delicate, interlacing fibrils were seen between the cells, and the whole appearance was identical with that of embryonic mesenchymal connective tissue.

The surface of these mesenchymal masses was generally lined by flattened cells with spindle-shaped or oval nuclei. In some areas, however, these lining cells underwent the same rounding and loosening changes as previously described (Fig. 59). The outline of the surface was either smooth, wavy, or papilliferous in appearance, and deep clefts were occasionally seen between adjacent polypoid masses (Fig. 58). In some sections it appeared that when these flattened lining cells made contact with other lining cells, whether of the normal air space epithelium (Fig. 60) or of other mesenchymal sheets (Fig. 58), the two layers fused, all trace of them was lost, and the original lining cells became indistinguishable from the other mesenchymal cells. No line of demarcation remained.

As the mesenchymal cells proliferated, it was observed that they were followed by a vascular outgrowth which provided a blood vessel core for the developing masses of tissue. In specimens which had not been

perfused, these vessels appeared as a complex honeycomb of spaces, some small and regular, others large and tortuous, with a lining of flattened cells (Fig. 61). Perfusion studies confirmed that the smaller were arterial and capillary in nature and that the larger were venous.

The histological changes which suggested the presence of oedema were difficult to assess in such a loose, open tissue. Although the oedematous changes seemed widespread in some mesenchymal masses, in others it appeared that they were largely confined to a zone beneath the surface lining. Here, the intercellular spaces contained not only the tiny fibrils previously mentioned, but also a considerable amount of amorphous granular material similar to that present in the air space. This material, precipitated protein, was not usually observed deeper within the mesenchymal masses.

In assessing the time at which the mesenchymal proliferation began after obstruction of the pneumatic foramen, there were two facts which needed to be taken into account. Firstly, depending on the age of the bird and the degree of pneumatisation, there were different amounts of subepithelial tissue present in the bones of different birds at the time of operation (Figs. 62,63). Secondly, the pneumatisation process in the experimental bone was arrested by blockage of the pneumatic foramen (see later). These two facts meant that at the time of sacrifice, the amount of subepithelial tissue present in the control bone, where air space development had been proceeding, bore no relationship to the amount present in the experimental bone either at the time of operation

Fig. 59. H.P. of newly-formed mesenchymal connective tissue. Some of the lining cells seen here are rounded. H & E x 500.

Fig. 60. Proliferating mass of mesenchyme approaching relatively normal lining. H & E x 200.

Fig. 61. Large vascular spaces in the proliferating mesenchyme. H & E x 500.

Fig.59

Fig.60

Fig.61

or at the time of sacrifice. One was, therefore, forced to regard only gross and obviously pathological changes as being significant, there being no valid controls against which to compare the changes.

On this basis, the development of the mesenchymal proliferative changes was observed to take place much more rapidly in partially pneumatised bones than in fully pneumatised bones. In the former, convincing evidence of pathological mesenchymal proliferation was found by 2-3 weeks after operation. In the latter, similar changes took 2-3 months to develop. Although it appeared that the changes may have occurred a little more rapidly after muscle graft operations than after the "Sevriton" block procedure, it was difficult to be certain about this point.

(iv) Fatty and myeloid changes.

Between 1-2 months after obstruction of the foramen pneumaticum, an alteration was observed in the character of cells in areas within the proliferating vascular mesenchyme. Some of the stellate mesenchymal cells became rounded and their cytoplasm took on a foamy appearance (Fig. 64). Their nuclei remained rounded or oval. The appearance was due to the intracellular deposition of lipoid material, as confirmed by staining frozen sections with "Oil Red O". As the change progressed, the mesenchymal tissue tended to take on a "wire-netting" pattern (Fig.65).

In other areas, a transition to true fat cells was seen. These cells were often found to appear in close relation to the blood vessels passing through the tissue (Fig. 66), and the fatty tissue, as first

Fig. 62. Fully pneumatised humerus. Normal air space lining. H & E x 200.

Fig. 63. Partially pneumatised humerus. Normal air space lining. H & E x 200.

Fig. 64. Foam cells within the proliferating mesenchyme. H & E x 200.

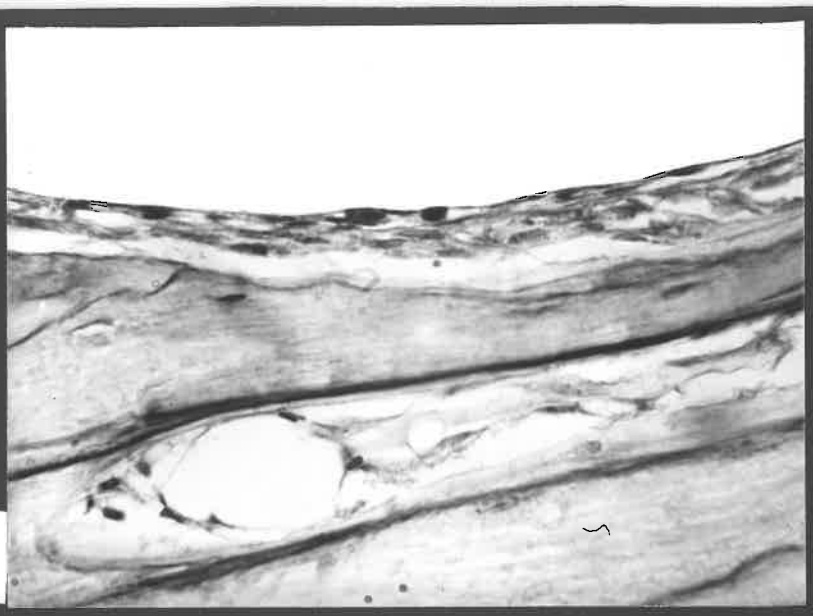


Fig.62

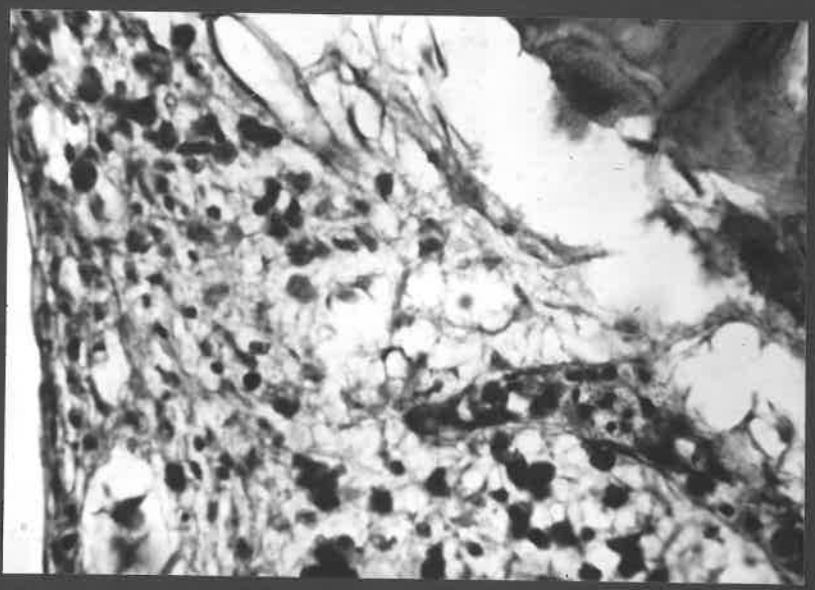


Fig.63

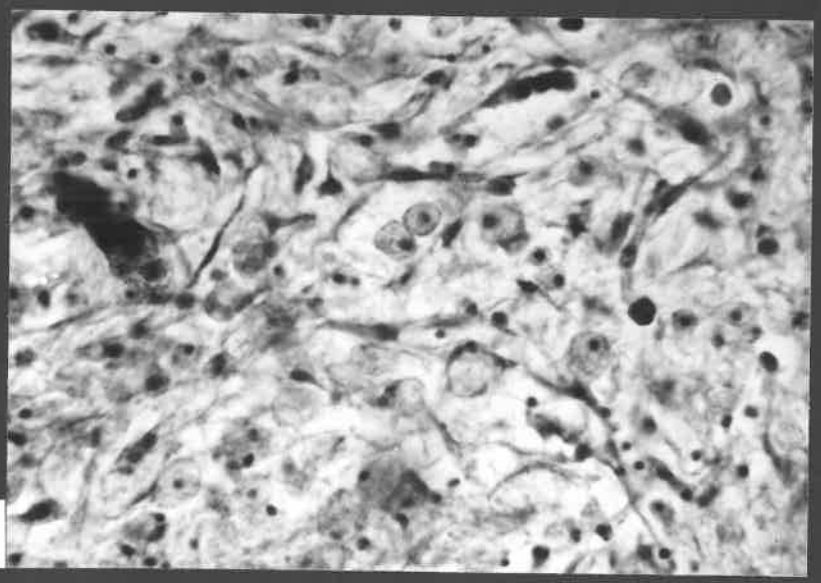


Fig.64

formed, was made up of small fat cells with marked variations in size and shape (Fig. 67). As the process continued, however, the individual cells became larger and more regular.

In association with the early stages of this fatty transformation, increasing numbers of free myeloid cells appeared. At first these tended to be scattered, but with the development of the fat cells they became aggregated into small islands. The burst of haemopoietic activity was short-lived, however. As the fatty tissue matured, the free myeloid elements became fewer in number and the overall appearance suggested a change to a slightly bizarre-looking fatty marrow (Fig. 68).

A reduction in vascularity occurred as the fatty tissue developed. The large, abundant, thin-walled, tortuous vessels of the mesenchymal connective tissue disappeared, and much smaller sinusoidal vessels remained. These could be filled by the venous perfusion technique and were related to the islands of myeloid cells. As the myeloid cells further decreased in numbers, however, even fewer vessels could be filled.

The first evidence of these changes was seen by one month post-operatively in partially pneumatised bones, and by two months post-operatively in fully pneumatised bones. The nature of the operative procedure, whether a muscle graft or a methyl methacrylate block, did not appear to be a significant factor in their time of onset. The process appeared to be a continuing one, and was observed to be taking place throughout the duration of the experiment.

Fig. 65. "Wire-netting" pattern in mesenchymal tissue, the result of lipoid deposition. H & E x 200.

Fig. 66. Formation of true fat cells in the mesenchymal tissue. The blood vessels are here filled with perfusion material. H & E x 100.

Fig. 67. Variations in size and shape of newly-formed fat cells. H & E x 200.

Fig.65

Fig.66

Fig.67

(v) Tissue reactions to cholesterol.

Shortly after the mesenchymal cells had begun their transformation into fat-containing cells, areas of cholesterol granuloma were observed (Fig. 69). The masses of granuloma tissue were found scattered throughout all regions of the obstructed pneumatic system of those bones which were affected. Their presence did not appear to depend on the nature of the method used to obstruct the pneumatic foramen, nor on the stage of pneumatisation reached prior to operation. Cholesterol granulomata were found in both partially and fully pneumatised bones after both muscle graft and "Servriton" block procedures from 2-3 months after operation. In general, the amount of granuloma tissue present was found to increase with the duration of surgical exclusion of atmospheric air, and appeared to be greater in fully pneumatised than in partially pneumatised bones.

The granuloma tissue itself was composed of numerous clefts containing doubly-refractile cholesterol crystals embedded in connective tissue. The clefts showed no regular pattern of alignment, and each tended to be bordered by occasional collagen bundles (demonstrated by Van Gieson's stain and Polarised light examinations). The connective tissue between the clefts was chiefly made up of cells of the mesenchymal type, many of which were foamy and heavily laden with fat (demonstrated by "Oil Red O" staining of frozen sections - Fig. 70). In addition, however, numbers of irregular, multinucleate giant cells (Fig. 71) were seen. No haemosiderin granules were observed in H. & E. sections or in

Fig. 68. Newly formed fatty marrow adjoining mesenchyme. The vascularity of the tissue decreases as the fatty transformation progresses. H & E x 100.

Fig. 69. Cholesterol granuloma.
H & E x 200.

Fig. 70. Cholesterol granuloma - frozen section stained with "Oil Red O" to show fat content. x 200.

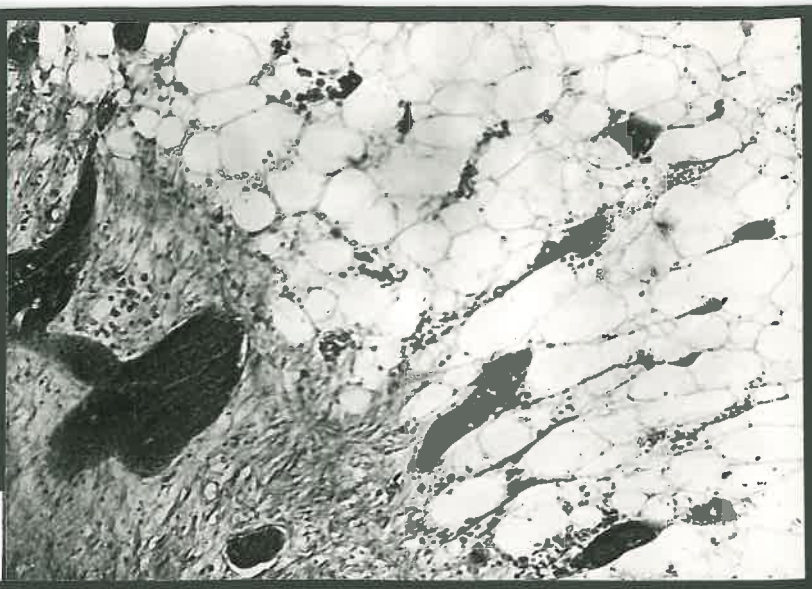


Fig.68

Fig.69

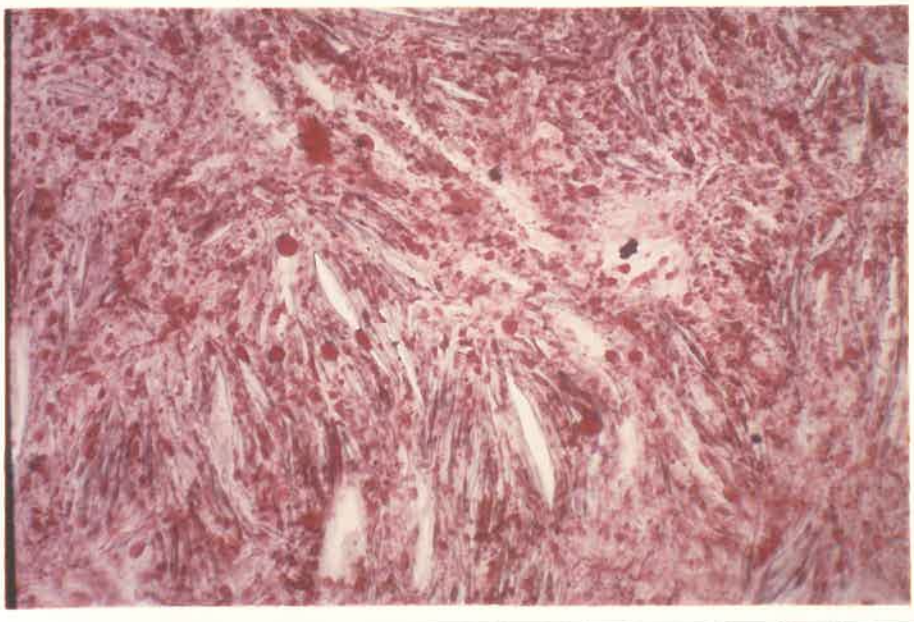


Fig.70

sections specifically stained for haemosiderin (Perl's stain).

The central area of the granuloma generally contained very few blood vessels. In perfused specimens, the majority of the vessels were found arranged around the periphery of the collection of cholesterol clefts (Fig. 72).

In many sections, it could be seen that most of thegranulomata were formed as a reaction to crystals deposited within the tissues following localized degenerative changes in the mesenchymal tissue. In others, however, they were obviously formed by the proliferation of connective tissue out into pneumatic spaces which had become filled with cholesterol crystals and fat. In both of these situations, strands of proliferating cells could be seen extending across the spaces and commencing to envelop the cholesterol crystals (Fig. 73). There was no histological evidence of old haemorrhage or suppuration in the tissues affected.

With further time, new bone formation was observed in some of the cholesterol granulomata. The bone was first laid down as narrow strips bordering the more central of the cholesterol clefts (Fig. 74). The deposition then continued between the clefts and extended peripherally (Fig. 75), replacing the bulk of the connective tissue of the granuloma (Fig. 76). Eventually the entire network of cleft-like spaces was seen to become encased in a block of new bone, the only remaining evidence of the original cholesterol granuloma being the persistence of crystal clefts in the centre of the bony mass (Fig. 77).

The pattern of osteoblastic activity during this process appeared to

Fig. 71. Cholesterol granuloma with numerous multinucleate giant cells. H & E x 200.

Fig. 72. Cholesterol granuloma. The vessels are filled with perfusion material. Few vessels are present in the midst of the cholesterol clefts. H & E x 100.

Fig. 73. Strands of connective tissue extending across a tissue space filled with cholesterol crystals. H & E x 200.

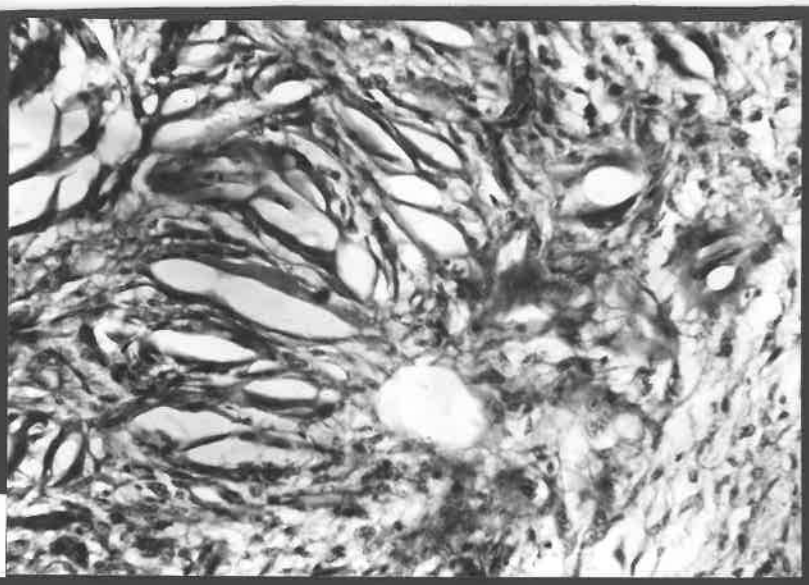


Fig.71

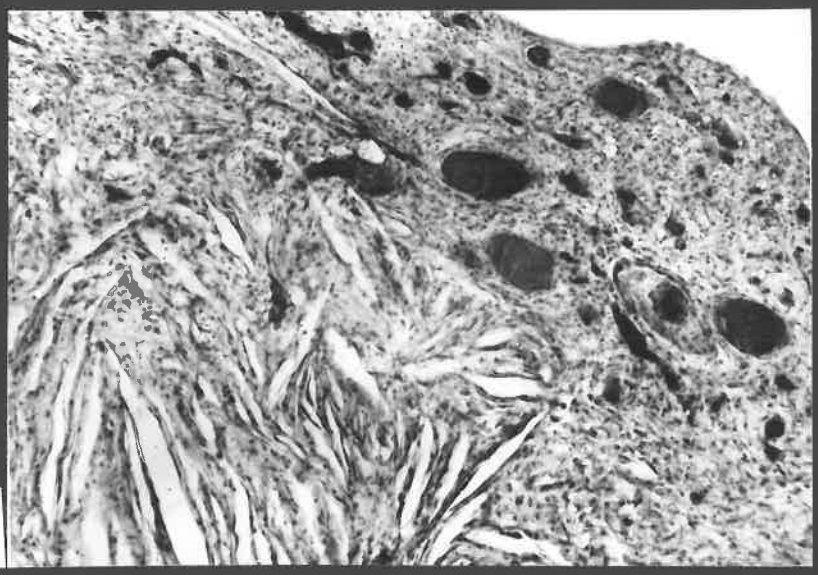


Fig.72

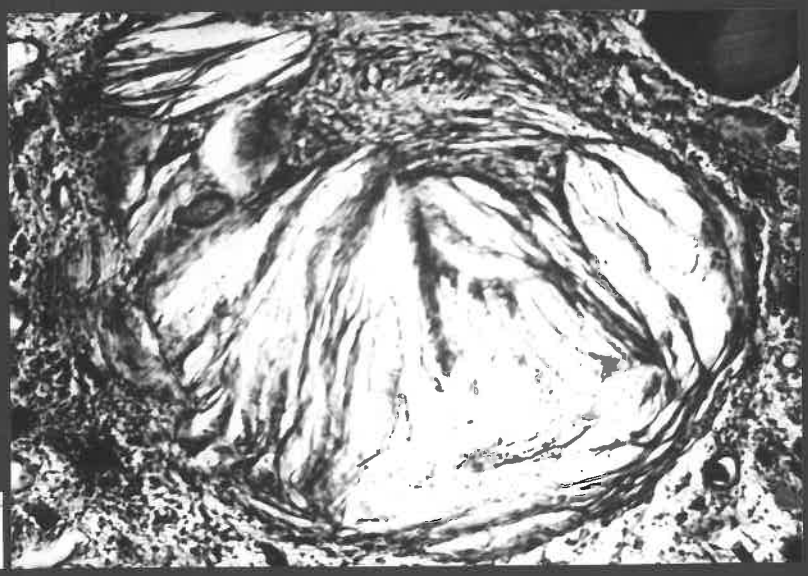


Fig.73

Fig. 74. New bone being laid down as strips between cholesterol clefts. H & E x 500.

Fig. 75. Peripheral extension of new bone deposition in a cholesterol granuloma. H & E x 200.

Fig. 76. Partial replacement of the connective tissue of a cholesterol granuloma by new bone formation. H & E x 100.

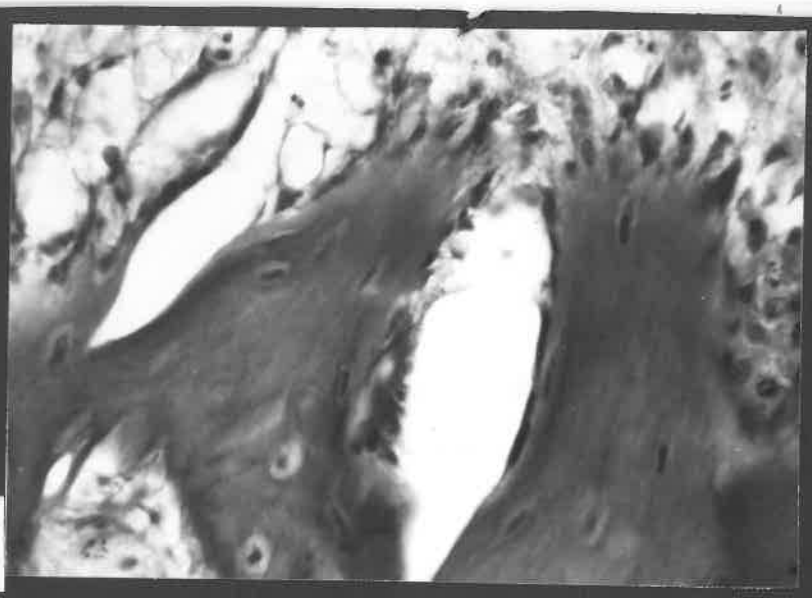


Fig.74

Fig.75

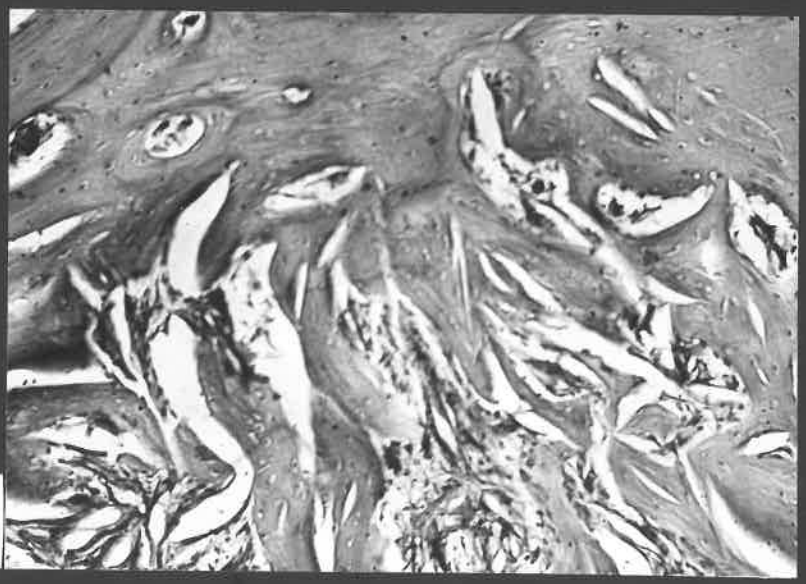


Fig.76

be preferentially arranged so that new bone was laid down in a direction away from the centre of the granuloma. Although large numbers of active osteoblasts were found on the expanding peripheral aspect of this newly-forming bone, neither osteoblasts nor osteoclasts were observed along the margins of the cholesterol clefts.

In the case of fully pneumatised bones, the deposition of new bone in areas of cholesterol granuloma was observed at 5 months, and subsequently, following the "Sevriton" block procedure, and at 4 months, and subsequently, following the muscle graft operation.

The phenomenon was less marked and less constant in bones of the partially pneumatised series. In this group, although new bone formation of the above type was found after the "Sevriton" block operation, no evidence of ossification could be found in any area of cholesterol granuloma following the muscle graft procedure. Even in the "Sevriton" series its appearance was inconstant. Here, it was first observed in one case after 3 months, but not in others until 6-7 months of complete obstruction had elapsed.

(vi) <u>New bone formation in mesenchymal tissue.</u>

After some months, the mesenchymal connective tissue began to exhibit areas of new bone formation as a result of the awakening of osteoblastic activity. This occurred in close relationship to blood vessels running through the tissue. Accordingly, two patterns of new bone formation were seen, and depended not only on the site at which the osteoblastic activity occurred, but also on the vascular pattern in the area.

The first of these patterns was observed in the midst of the sheets of vascular mesenchymal tissue where isolated islands of new bone appeared (Fig. 78) and enlarged in various directions (Fig. 79). Although these showed no cement lines initially, they did so after fusion with adjacent islands of new bone, and after subsequent enlargement and remodelling (Fig. 80). In this way, a bizarre reticular pattern was established which enclosed small spaces containing blood vessels and strands of mesenchymal tissue (Fig. 79). In laying down this bony pattern, the osteoblastic activity was closely related to blood vessels which were found ramifying in a random and tortuous fashion through the proliferating mesenchymal tissue.

The second arrangement was observed just beneath the lining of the air space. In this region, plates of new bone were laid down in an orderly fashion immediately deep to and parallel with the epithelium (Figs. 81a, 81b). In this situation, the pattern of osteoblastic activity was again closely related to the complex network of vessels found lying in the mesenchymal tissue in a plane more or less parallel with and subjacent to the lining epithelium itself (Fig. 82).

These patterns of new bone formation were found in one case (C.B. 18, muscle graft on a fully pneumatised bone) after two months, but in general were not present until 4-5 months after operation. There did not appear to be any great difference between the partially and the fully pneumatised series regarding the time of onset of the change, but the impression was that it occurred a little earlier, and to a more

Fig. 77. Late stage of bone deposition in a cholesterol granuloma. The only evidence of the origin of the new bone is the persistence of crystal clefts. H & E x 100.

Fig. 78. Island of new bone deposition in mesenchyme in relation to a small vessel filled with perfusion material. Remodelling is starting. H & E x 500.

Fig. 79. Enlarging areas of new bone formation in mesenchymal tissue. The pattern is laid down around the ramifying blood vessels, here filled with perfusion material. H & E x 500.

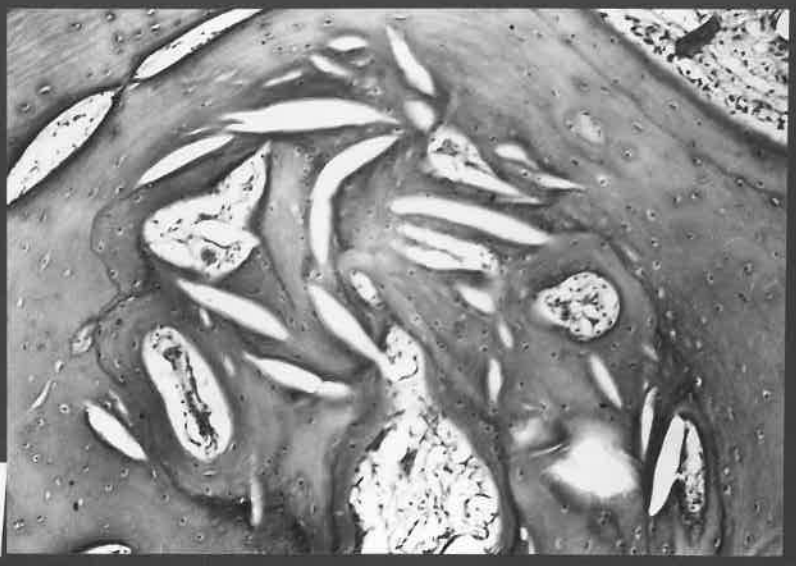


Fig.77

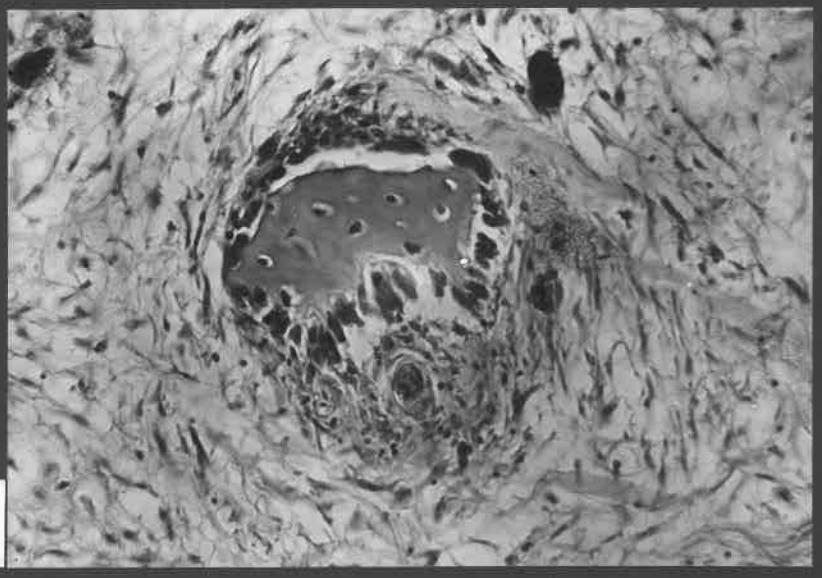


Fig.78

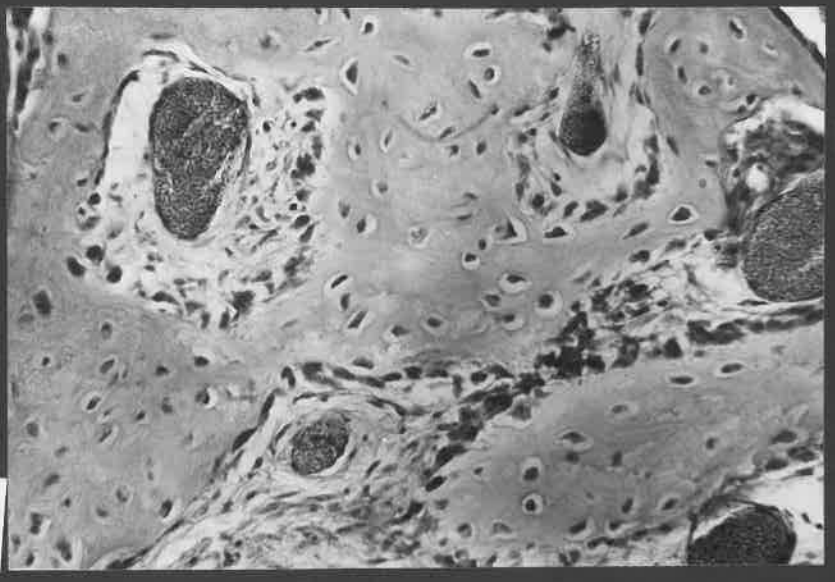


Fig.79

Fig. 80. Further enlargement and remodelling in bone deposited in mesenchymal tissue. H & E x 200.

Figs 81(a), 81(b). Bony plates laid down just beneath the epithelial lining. H & E 81(a) x 100, 81(b) x 200.

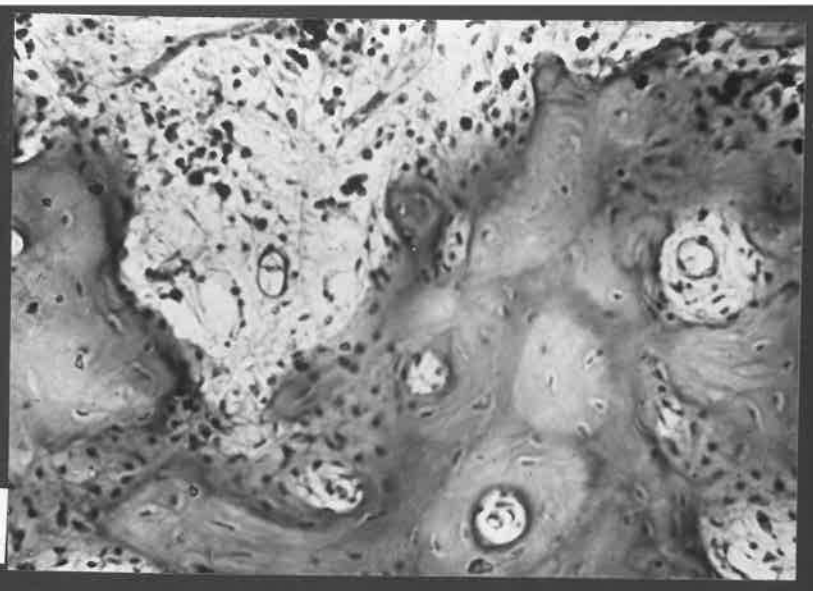


Fig.80

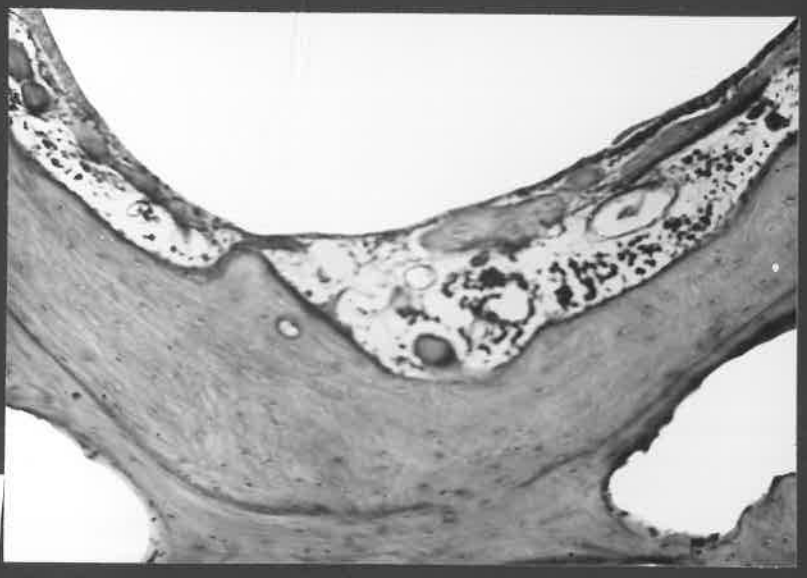


Fig.81a

Fig.81b

marked degree, after the muscle graft operation than after the "Sevriton" block operation.

(c) The Bone substance itself.

As pneumatisation proceeds, a progressive bony remodelling is observed. Both osteoblastic and osteoclastic activity are seen, but the latter is the more dominant and is responsible for the thinning and disappearance of the trabeculae and the excavation of the endosteal surface of the cortex. The osteoclastic activity is directed towards those trabeculae and endosteal areas of the bone cortex which are adjacent to or enveloped by the advancing zone of subepithelial mesenchymal tissue. (Fig. 36).

Following operative blockage of the foramen pneumaticum, new bone formation was seen in the area of the surgical intervention. This was in the nature of a local bone healing process and was the expected reaction to local damage to trabeculae and cortical bone in the region of the operation.

However, a much more interesting phenomenon was observed in other areas of the pneumatic system remote from the site of surgical interference. In pneumatising bones as early as the first week or two after operation, osteoclastic activity decreased and osteoblastic activity increased along bony surfaces adjoined by the mesenchymal tissue. New bone was not laid down on bone surfaces adjoined by fat cells (Fig. 83).

As a result, the bone removal process slowed and new bone was progressively laid down on the existing trabeculae, so thickening and

strengthening them (Fig. 84). In bones which were fully pneumatised at the time of the operative procedure, the changes were less dramatic and took longer to develop. Nevertheless in both series, the formation and remodelling of the new bone, as evidenced by the great osteoblastic activity, the presence of osteoclasts (Figs. 85, 86), and the development of often complex patterns of cement lines (Figs. 87a, 87b), eventually occurred throughout the entire air space system.

It should be emphasised that none of these changes, nor any previously mentioned as occurring in the experimental series, bore any resemblance to the peculiar type of medullary new bone formation normally occurring in the humerus of the laying hen (Fig. 88). This latter type of new bone deposition is physiological and quite specific. The bone is laid down in a reticular pattern (Fig. 89) which is strikingly based on the normal sinusoidal vascular pattern (Fig. 90). It is unusually basophilic in haematoxylin and eosin sections.

(d) The bone marrow.

The bone marrow already present in the bones prior to operation (Figs. 91a, 91b) did not appear altered in any way as a result of the procedure. The fatty tissue, the haemopoietic elements, and the sinusoidal vascular pattern appeared to remain unchanged on histological examination.

A possible exception to this was seen when comparing the width of the advancing subepithelial connective tissue zone in experimental bones with control bones at an equivalent stage of pneumatisation. It was

Fig. 82. Blood vessels, filled with perfusion material, in the subepithelial mesenchymal tissue. These run parallel with the lining of the air space and are related to the deposition of subepithelial bony plates. H & E x 100.

Fig. 83. New bone formation is seen where mesenchymal tissue adjoins the endosteal layer, but not where fat cells rest directly on the old bone. H & E x 200.

Fig. 84. New bone deposition on pre-existing trabeculae. H & E x 200.

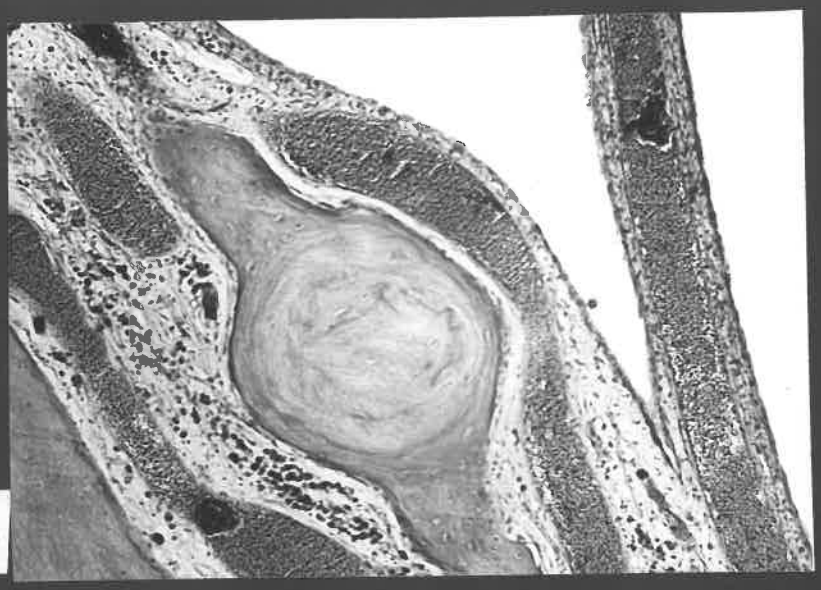


Fig.82

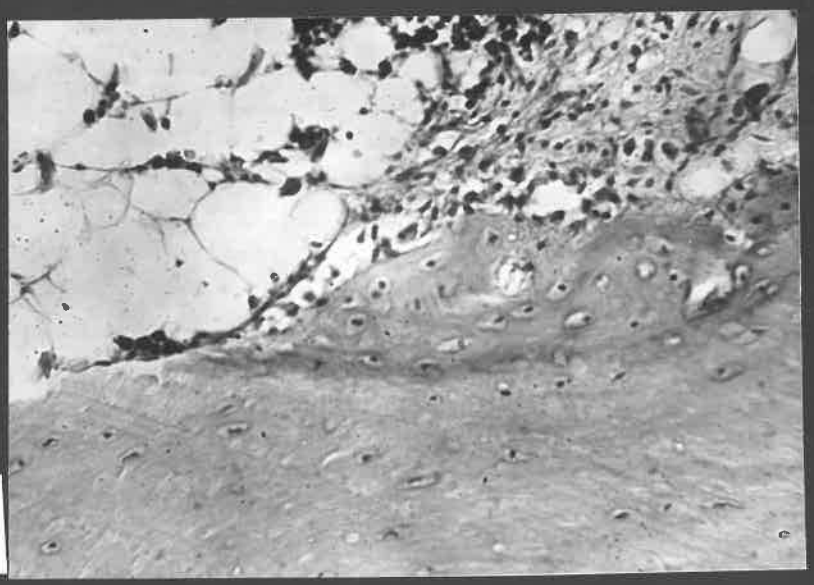


Fig.83

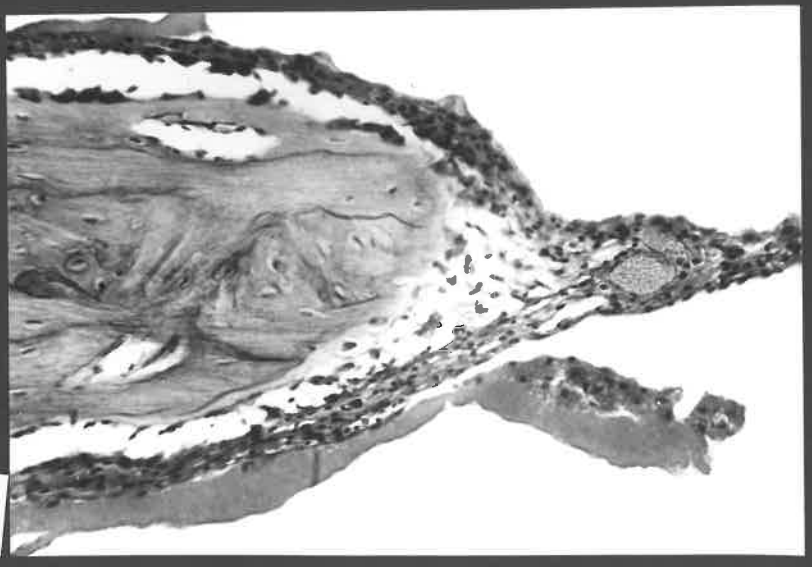


Fig.84

Fig. 85. The connective tissue island shows osteoclastic activity at one end and osteoblastic new bone formation at the other. H & E x 100.

Fig. 86. T.S. through an osteoclastic cutting cone. H & E x 200.

Fig. 87(a) Complex cement line patterns are often seen in the longer term bones. H & E x 200.

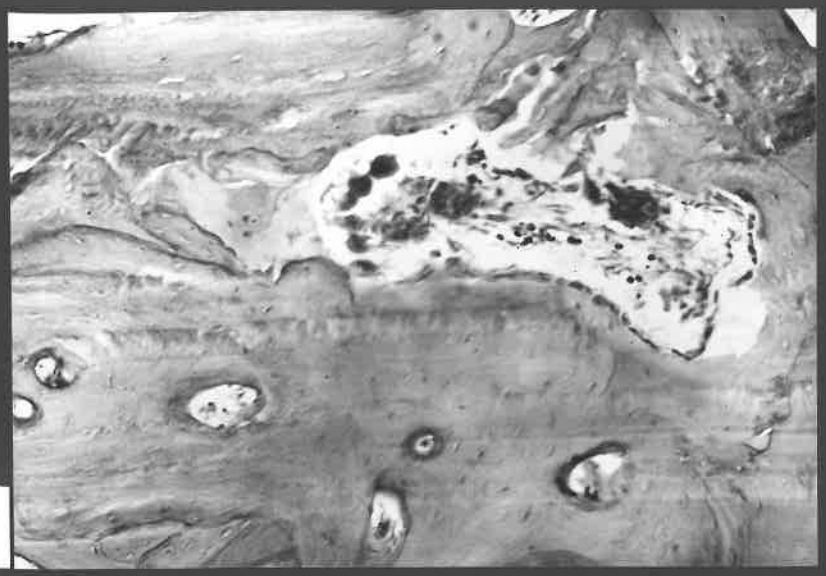


Fig.85

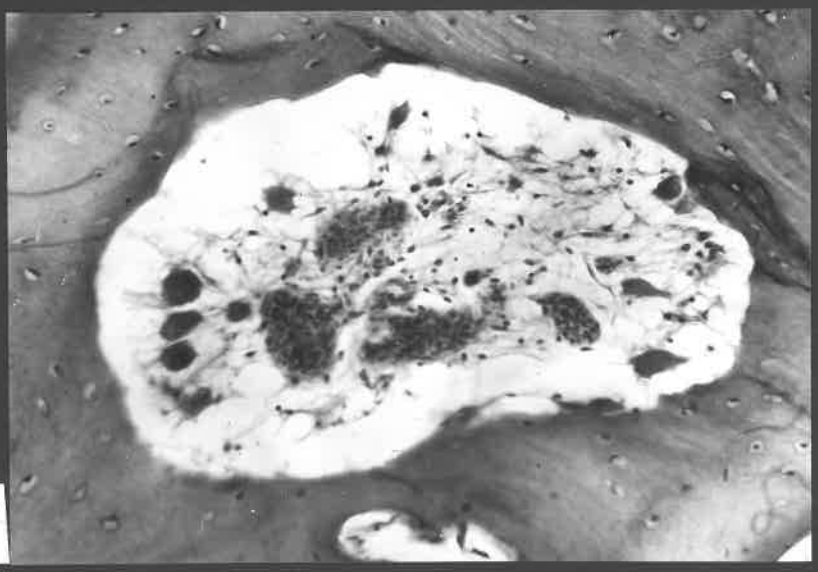


Fig.86

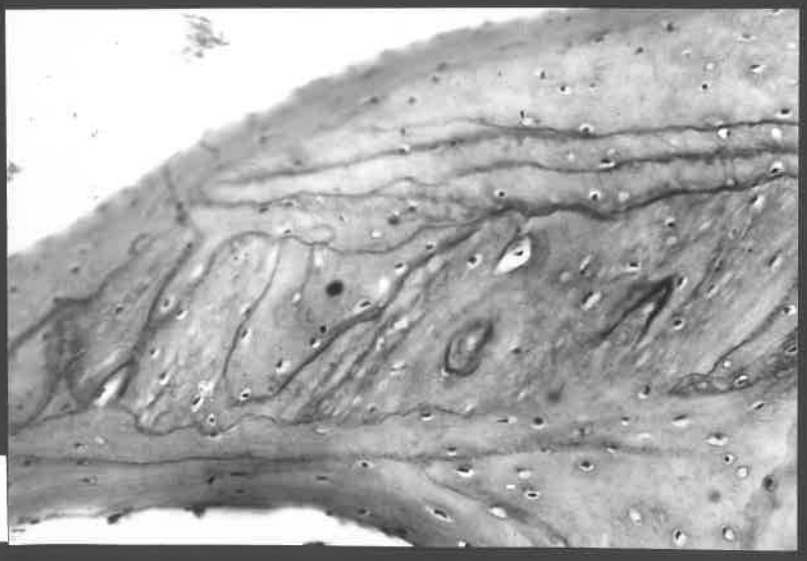


Fig.87a

Fig. 87(b). Complex cement line pattern in a long term bone. H & E x 200.

Fig. 88. Physiological medullary new bone formation in the humerus of the laying hen. The new bone is darker in colour (basophilic) than the normal trabeculae. H & E x 100.

Fig. 89. Reticular arrangement of physiological new bone deposited in the marrow. The pattern is similar to that of the sinusoidal network. H & E x 100.

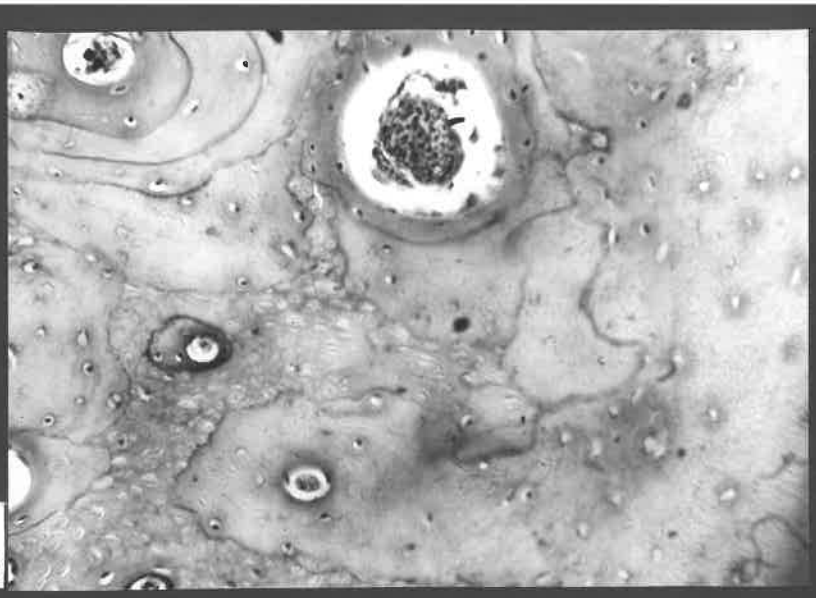


Fig.87b

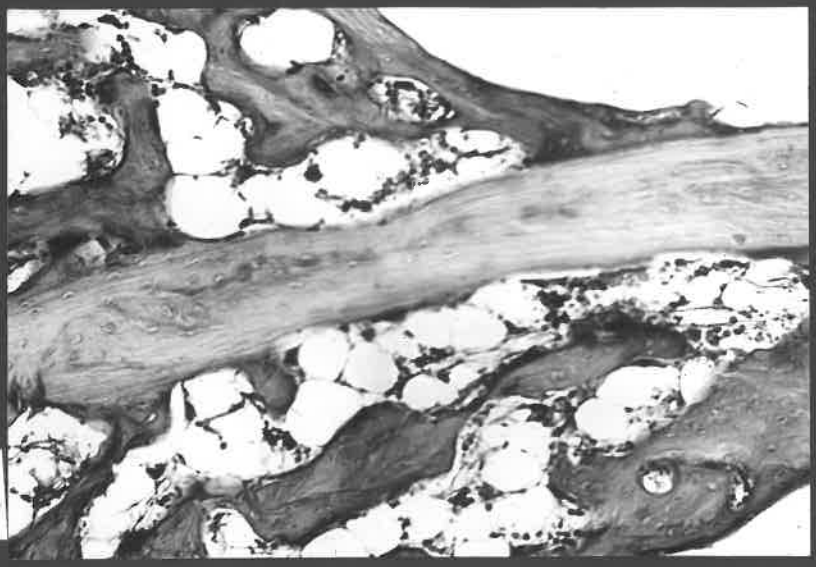


Fig.88

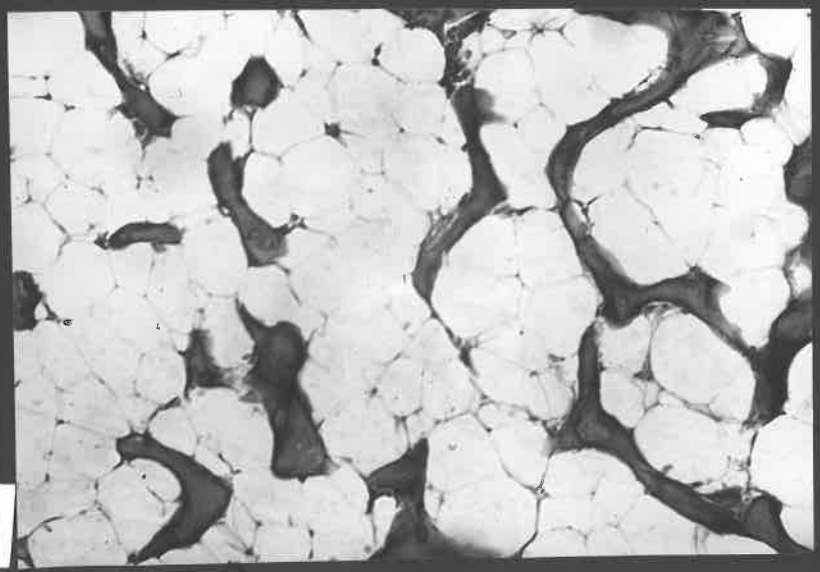


Fig.89

Fig. 90. Normal sinusoidal pattern of the bone marrow of Gallus domesticus. The sinusoids are filled with perfusion material. H & E x 100.

Figs. 91(a), 91(b). Persisting marrow islands in fully pneumatised humeri. H & E x 100.

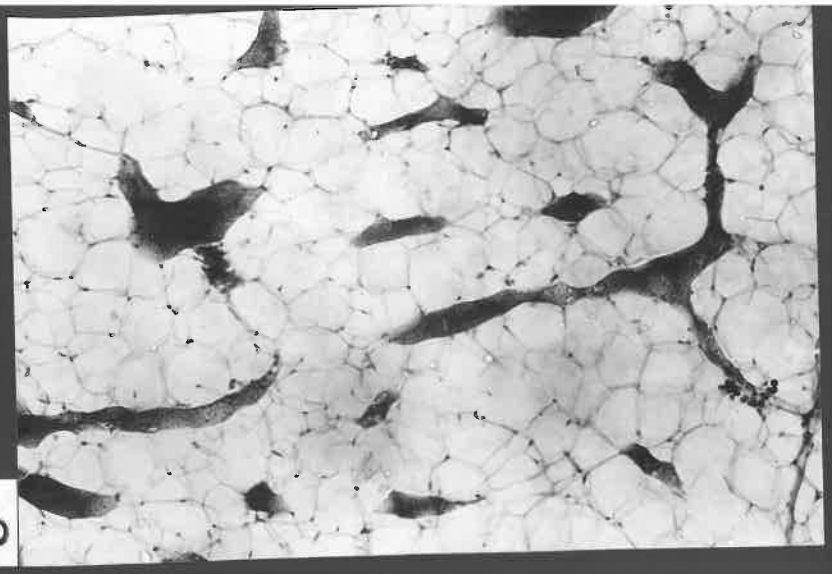


Fig.90

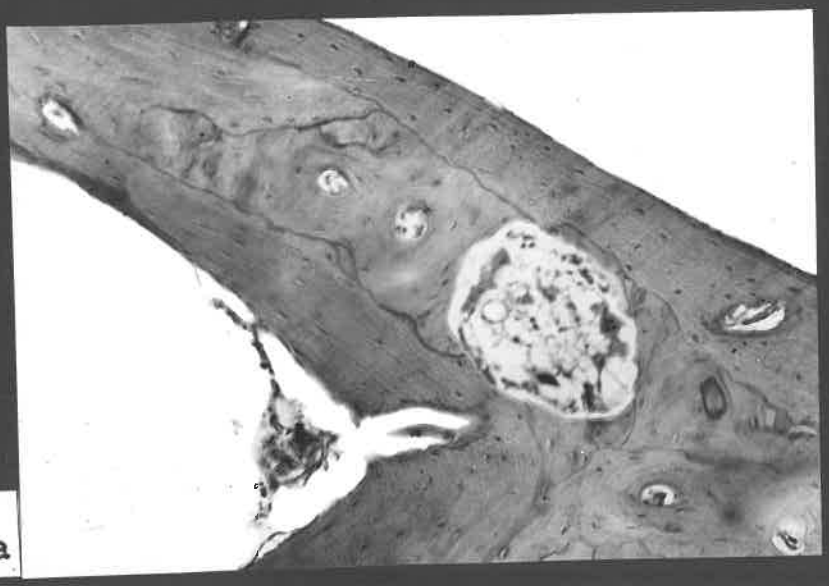


Fig.91a

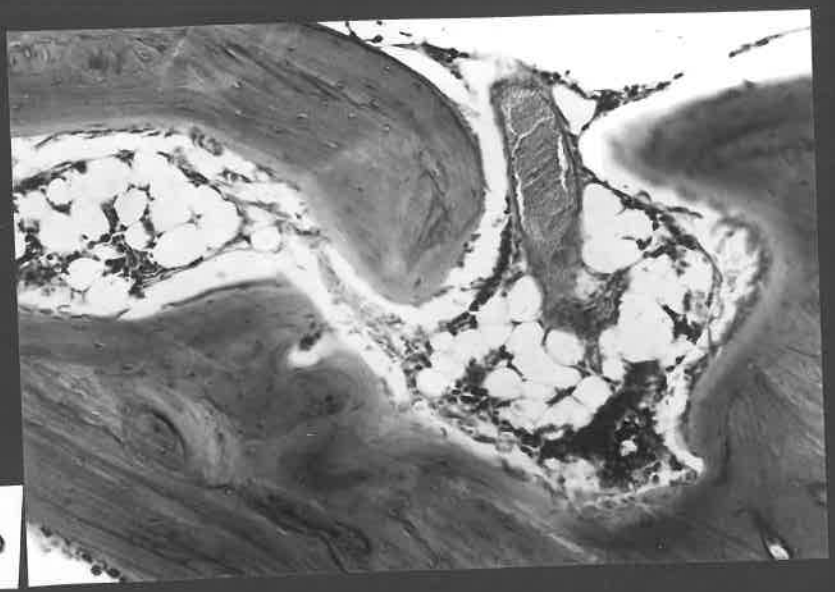


Fig.91b

observed that in experimental bones there was a slight increase in the width of this zone at the expense of a small portion of the subjacent marrow. The majority of operated bones showed this feature, but there was no evidence that it progressed after the first one or two weeks.

(e) The pneumatic space itself.

One week after obstruction of the foramen pneumaticum, the air space still contained a moderate number of red cells (nucleated in birds) which had been released into the space during the operative procedure, together with a small amount of eosinophilic, granular, proteinaceous material. Later, as the epithelial changes became established, the space also contained an increasing number of pale, foamy, rounded cells with small, dense nuclei.

The numbers of red cells in the space tended to decrease with the duration of the experiment, and very few were seen in the later stages of any series. There was certainly no evidence of any significant post-operative haemorrhage into the pneumatic system.

The amount of granular, protein material in the air space steadily increased with the duration of the experimental blockage, and, after 2-3 months, this amorphous material also exhibited numerous clefts when examined histologically after fixation. At first it was considered that these were artefacts of preparation and sectioning. However, when fresh material was viewed under polarized light many of these clefts were seen to contain doubly refractile crystals of cholesterol. Fat globules were also seen.

By comparing the pre-operative size of the pneumatic space, as viewed radiologically, with the post-operative size, as seen histologically in both experimental and control bones, it was obvious that the process of pneumatisation ceased following the exclusion of air from the pneumatic system. However, it was not possible to make an exact comparison between the pre and post-operative dimensions of the space because

(i) The two methods of assessment, radiological and histological, were different and rather inexact in themselves,

(ii) The continued growth of the bone, both in length and diameter, of necessity altered the size of the air space and the ratio between this and the total bone length.

Accordingly, it was not possible to clarify whether enlargement of the pneumatic system ceased at the time of blockage of the foramen pneumaticum, or whether it continued for a short time before coming to a halt.

In any event, it was established that the existing air space was reduced in size by space-filling oedematous changes following the operative intervention, and that the volume was soon further diminished by subepithelial mesenchymal proliferation and the associated fatty changes. Fusion of these mesenchymal masses with each other and with areas of normal lining tissue, together with connective tissue growth into and across spaces filled with protein material, cholesterol crystals, and fat, caused the obliteration of many regions of the pneumatic system. New

bone was laid down on pre-existing trabeculae and on the endosteal surface of the bone cortex, in addition to being deposited in areas of cholesterol granuloma and mesenchymal connective tissue. These changes have already been described in detail.

When viewed macroscopically after fixation and decalcification, the original air space in the longer term bones was found to be reduced to a scattered collection of small, fluid-filled, cystic spaces amid this mass of newly formed tissue. No specimen in the series ever showed total histological obliteration of all areas of the pneumatic system, but it is probable that this would have occurred eventually if the experiment had been carried on for a longer time.

5. Vascular changes.

The initial vascular changes of dilatation and congestion which occur as a result of simple blockage of the foramen pneumaticum have already been dealt with. However, in association with proliferative changes in the mesenchymal tissue and with the appearance of cholesterol granuloma tissue and new bone, a host of new vessels appear. The histological appearance and distribution of these vessels have been described in previous sections, but it remains to discuss the changes in the overall vascular pattern as studied radiologically and by the Spalteholz technique in perfused experimental bones.

(i) Arterial pattern.

The outstanding feature was the appearance of a host of new vessels which pursued an extremely tortuous and irregular course. They

showed as complex loops and spirals which often appeared to become tangled in a confused network (Fig. 92). These vessels arose from and were superimposed on the basic arterial pattern which itself became progressively more tortuous, but tended to remain in the position to which it was displaced by the process of pneumatisation. The resultant appearance was quite distinctive and in sharp contrast to the orderly arterial distribution in marrow-containing portions of the bone (Fig. 93).

In the proximal third of the humerus, an additional vascular alteration resulted from the operative procedure which, of necessity, interfered with the blood flow through the foramen pneumaticum. However, alternative vascular pathways were abundant. Both the proximal accessory nutrient vessels and the ascending division of the nutrient artery opened up and maintained the blood supply to this area of the bone. The distal two thirds of the humerus were not, of course, involved by the adjustment.

(ii) Venous pattern.

Because of the greater capacity of the venous system, the changes in the venous pattern were even more dramatic. A profusion of new veins was seen. These ran in all directions forming a densely interlaced meshwork of vessels, the pattern of which was quite different from the tortuous appearance of the arterial system (Fig. 94). The new vessels were often of considerable size, although in many areas a sinusoidal arrangement was seen. In the lower two thirds of the bone, collecting veins arose from the meshwork and, after running in a generally

Fig. 92. Humerus, fully pneumatised. Arterial perfusion, several months after a "Sevriton" block. The arteries are extremely tortuous and irregular in their course. Radiograph.

Fig. 93. Humerus. Arterial perfusion, several months after a "Sevriton" block. The bone was half pneumatised at the time of operation. The distal half is marrow-containing with an orderly arterial pattern, the proximal half shows the typical tortuosity of vessels. Radiograph.

Fig. 94. Humerus, fully pneumatised. Venous perfusion, several months after a "Sevriton" block. The veins form a complex, interlaced meshwork. Radiograph.

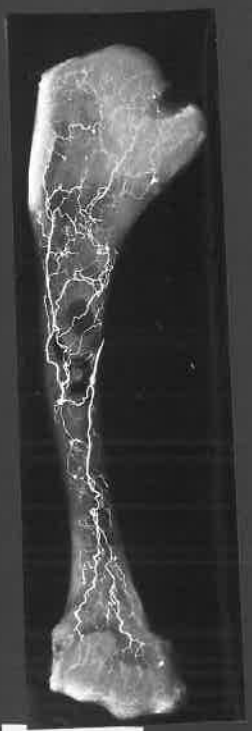


Fig.92

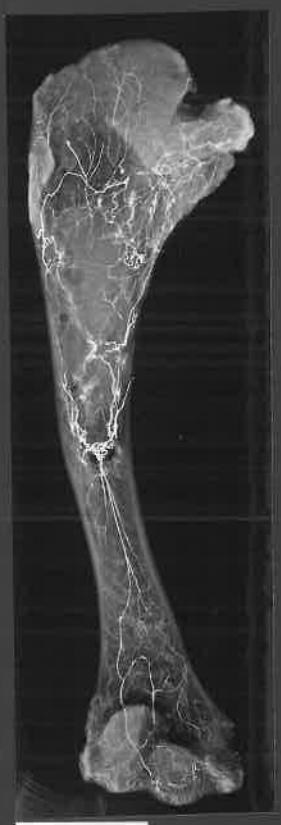


Fig.93

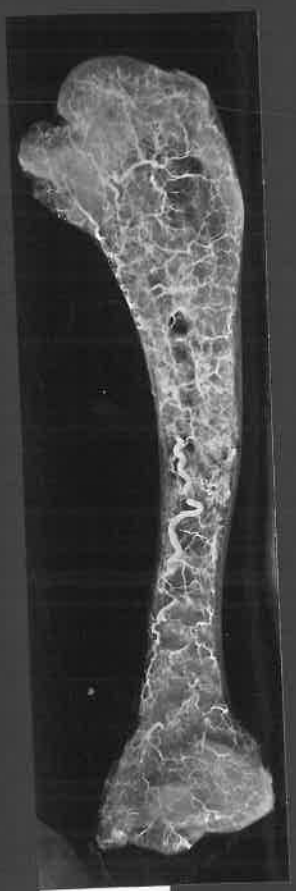


Fig.94

transverse or oblique direction, joined the rather tortuous medullary vein.

At each end of the post-operative humerus, the accessory veins became enlarged and were more prominent than normal. This was especially marked in the proximal third of the bone where the vein of the pneumatic canal had been interfered with by blocking the pneumatic foramen. In this region, the venous drainage was almost entirely taken over by the accessory veins which were considerably dilated and passed out of the bone through enlarged foramina. However, additional venous channels also passed downwards to communicate with the medullary vein itself, and it was observed in Spalteholz specimens that portion of the proximal division of the medullary vein had also opened up.

VI. DISCUSSION.

1. Preliminary Vascular Studies.

In the present investigation, it was found that a preliminary study of the arterial supply of the wing of the domestic fowl was required because of the absence of a sufficiently accurate or detailed account of this subject in the literature, and because such information was essential for the planning and interpretation of the experiments. By means of both radiological and dissection techniques, it was possible to demonstrate the origin, course, and distribution of the arteries of the wing proximal to the carpus, and of the arteries supplying the humerus in particular.

It was observed that apart from the periosteal vessels, the humerus is chiefly supplied by the nutrient artery which enters the bone at the junction of its middle and proximal thirds. In addition, this is supplemented, in the adult bone, by the artery of the pneumatic canal and by numerous small vessels entering at each end of the bone. At the proximal end, these vessels arise mainly from the artery of the pneumatic canal, the nutrient artery of the humerus, and the circumflex artery, all of which are derived from the humeral artery. At the distal end of the bone, they arise mainly from the supratrochlear artery and the posterior terminal branch of the profunda brachii artery.

In planning procedures designed to exclude air from the pneumatised humerus, it was considered desirable to ensure that these vessels were preserved. In this way, it was hoped to maintain the blood supply of the entire bone and thereby avoid ischaemia as a complicating factor in

the interpretation of the pathological findings. However, although it was possible to preserve the nutrient artery and the small vessels of the bone ends, it was found that damage to the artery of the pneumatic canal was generally unavoidable.

After first establishing the pattern of the intra-osseous blood vessels of the normal marrow-containing ulna, an investigation of the course and distribution of the intraosseous vessels of the humerus at different stages of pneumatisation was therefore carried out. This study demonstrated that although the vein of the pneumatic canal is present in the bone prior to air space formation, the artery of the pneumatic canal enters during the pneumatisation process. Further, in the pneumatised bone, its area of ramification is limited to the proximal third of the shaft, and this distribution is overlapped not only by the vascular distribution of the ascending division of the nutrient artery, but also by the distribution of the accessory nutrient vessels of the proximal end of the bone. On anatomical grounds there was, therefore, no reason why damage to the artery of the pneumatic canal should prove a significant factor.

Vascular studies on bones following the operative interventions confirmed that the blood supply of the proximal third of the humerus was adequately maintained by these routes and by the opening of further alternative anastomotic pathways. The vascular supply of the remainder of the bone was not involved by the changes.

2. Effects of Surgical Obstruction of the Foramen Pneumaticum - factors

involved.

As a result of surgical blockage of the foramen pneumaticum of both partially and fully pneumatised humeri in the domestic fowl, using either a muscle graft or a block of methyl methacrylate, a progressive series of changes was observed which may be summarised as follows:-

(i) dilatation and congestion of vessels situated in the sub-epithelial tissues.

(ii) fluid transudation into the pneumatic system.

(iii) oedema of the lining tissues of the air space.

(iv) changes in the normally flat lining epithelium.

(v) proliferation of the mesenchymal tissue and the formation of new vessels.

(vi) the development of cholesterol granulomata.

(vii) Myeloid changes in the mesenchymal tissue.

(viii) new bone formation occurring in four distinct patterns:-

a) new bone deposition on pre-existing trabeculae.

b) reticular new bone formed in the mesenchymal masses.

c) trabeculae of new bone laid down parallel with and just deep to the lining epithelium.

d) new bone deposition in the cholesterol granulomata.

(ix) progressive obliteration of the pneumatic system by these changes.

In no specimen included in the series was there any evidence of infection or ischaemic bone damage. Other factors must, therefore, be

considered when discussing the development of the observed changes.

(a) Local surgical trauma.

The local surgical interference at the foramen pneumaticum was limited to gentle curettage of the pneumatic fossa in the case of the methyl methacrylate procedure. In the case of placement of a muscle graft, the interference was more extensive. A small portion of cortical bone was removed and the trabeculae in the immediate vicinity of the opening were fractured. In both these procedures, there was an unavoidable local reaction which involved an increase in osteoblastic activity as part of a repair phenomenon.

In bones which had been operated upon, but in which the muscle graft or methyl methacrylate block had become dislodged so that exclusion of air from the pneumatic system was ineffective, the operation area exhibited the changes mentioned above, but all other regions of the air space remained completely normal. The alterations which occurred in the air space under the conditions of the experiment could not, therefore, be attributed to the effects of local surgical trauma at the foramen pneumaticum. However, one factor may have been of significance. The surgical procedures inevitably caused the release of blood into the air space, and in bones where the block had been effective, there was no pathway by which this could escape. The possible importance of this will be dealt with later.

(b) Tissue reactions to the obstructing material.

Under the conditions of the experiment, the tissue reaction to

methyl methacrylate was minimal, and strictly localized to the immediate vicinity of the foreign material. In bones where the methacrylate was present in, but did not completely obstruct, the foramen pneumaticum, no alterations were observed in the pneumatic system. In cases where a muscle graft had been used and was deficient or had been dislodged so that a communication with the atmosphere persisted or had reformed, the lining of the pneumatic system also remained unchanged.

The experimental changes could, therefore, not have been due to reactions to the materials used for obstruction of the foramen pneumaticum.

However, one factor may have been of significance. In the majority of cases, a large portion of the muscle graft, chiefly that contained inside the air space, was seen to disappear, presumably because of atrophy and degeneration following interference with its blood supply and nerve supply. This loss of tissue must have been associated with the release of tissue break-down products into the enclosed air space. The possible importance of this will be dealt with later.

(c) Development of negative pressure.

It is generally accepted that fluid transudation is influenced by the following factors:-

(i) a reduction in the plasma osmotic pressure.

(ii) an alteration in the pressure relations inside and outside the capillary.

(iii) an increase in the filtering surface.

(iv) increased capillary permeability.

(v) obstruction of lymphatic channels (Best & Taylor 1961).

The first of these factors does not apply in the present instance, but the others merit consideration.

In an air-containing bone, mechanical blockage of the sole pathway of communication of the pneumatic system with the atmosphere prevents air from entering the air spaces. It is widely accepted (p. 58) that when this occurs, the trapped oxygen is absorbed and a negative pressure is created within the air space. This represents a reduction in the extravascular pressure and leads not only to the transudation of fluid into the lining tissue and into the air space, but also to capillary dilatation which further increases the filtering surface.

Although oxygen levels and pressure variations were not recorded in the present study, it is almost certain that the development of a negative pressure by a mechanism similar to this was one of the main factors in the development of oedema, fluid transudation into the air space, and congestion and dilatation of blood vessels.

(d) Increase in capillary permeability.

Although not specifically measured in the present investigation, two other factors may have been involved in the escape of fluid into the air space and tissues of the experimental humerus. It has already been mentioned that, under the conditions of the experiment, free blood and the products of tissue breakdown accumulated in the enclosed air space, and that the oxygen tension in the pneumatic system was reduced following

blockage of the foramen pneumaticum.

It is probable that these factors may have led to an increase in capillary permeability and further facilitated the fluid transudation.

(e) Role of the lymphatics.

Eggston and Wolff (1947) described the presence of lymphatic vessels in the mucosa of the middle ear of man, but little is known about their presence in the mastoid air cells. Yoffey and Courtice (1956) found no evidence of them in normal bone, so that it must be presumed that if they exist in the pneumatised humerus, they must pass through the foramen pneumaticum, having entered the bone from without. Disruption and consequent blockage of such vessels by operative procedures at the foramen pneumaticum would be inevitable. There was certainly no conclusive evidence that lymphatic channels, if in fact present, were functional in the bones examined.

Accordingly, the absence of effective lymphatic absorption from the air space and its lining tissues must be considered as a possible factor in the accumulation of the transudated fluid and its constituents.

(f) The protein content of the transudate.

In 1934, Drinker, Field and Homans (1934) demonstrated that where oedema fluids collect and are low in protein, tissue growth does not occur, but that where the fluids are rich in protein, an excellent "culture medium" is provided for the growth of connective tissue cells. In their experiments on dogs, they showed that the overgrowth of connective tissue becomes noticeable after a protein-rich lymphoedema has been present for

two months. Infection was excluded as a factor in the development of this phenomenon.

Homans, Drinker and Field (1934) found the same phenomenon in human elephantiasis. Samples of tissue fluid were taken at intervals and showed steadily increasing amounts of protein. As the tissue fluid approached the character of blood serum, the connective tissues showed evidence of active proliferation. Infection aggravated the reaction, but was thought not to be the prime cause.

Although the protein level is probably not the only factor involved in this phenomenon (Willmer 1960), a similar mechanism appears to have been operative in the pneumatised humerus following surgical closure of the pneumatic foramen. The protein content of the transudate filling the obstructed pneumatic system was initially much lower than that of blood serum, but with an increase in the duration of obstruction it was observed to rise markedly. This rise more or less coincided with the onset of proliferative changes in the mesenchymal tissues.

The protein rise was probably the result of a combination of factors. Free blood released into the air space at operation, degenerative changes in the muscle graft, and the loss of lining cells into the cavity of the air space, almost certainly provided some of the protein. However, it is known that protein molecules escape through capillary walls and re-enter the blood stream only by way of the lymphatics (Drinker and Field 1931). In the pneumatised humerus under the conditions of the experiment where there is no effective lymphatic function, the most important factor

must therefore have been the continued escape of protein from the capillaries in the absence of any effective means of reabsorption.

(g) The deposition of cholesterol crystals.

Cholesterol granulomata were first described in the ear by Manasse (1917). Since that time many workers (Birrell 1956) have confused the condition with that of cholesteatoma. However, the two have been shown to be separate, though often combined, entities (Friedmann 1959).

In pneumatised bones, the pathogenesis of the granuloma has been the subject of dispute for some time. It has usually been considered that the crystals of cholesterol are deposited at the site of haemorrhage (Simonetta 1949), or in the course of chronic infection (Grippaudo 1959). However, the presence of altered blood is not an invariable finding (Rewell 1963), and it has been stated that cholesterol granuloma is never seen in experimentally infected ears (Friedmann 1959). Friedmann (1959) was able to reproduce the condition only after repeated injections of sterile suspensions of cholesterol into the middle ear of the guinea pig. Cholesterol granulomata were found only in non-infected ears, usually in the region of the needle track or in the more protected parts of the bulla.

In the present study, it was observed that Cholesterol granulomata were formed as a reaction to the presence of cholesterol crystals (Fig.95) deposited both in the air space and in the tissues. The deposition was first seen after 5-6 weeks of obstruction of the foramen pneumaticum. The first evidence of granuloma formation was not found, however, until

Fig. 95. Cholesterol crystals lying free in fluid-filled air space. Fresh specimen, photographed under polarised light.

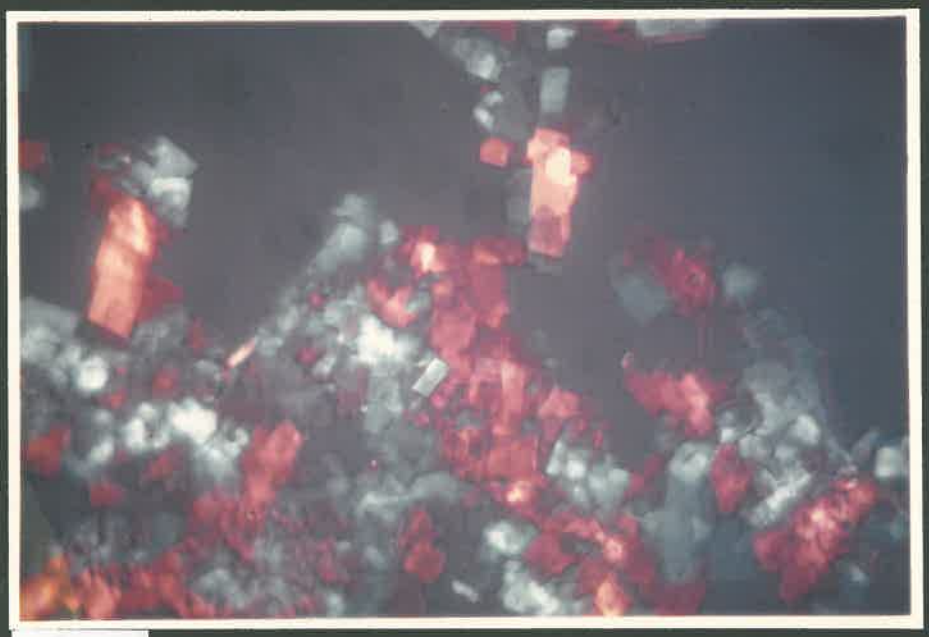


Fig.95

6-8 weeks later.

There was no evidence that infection or haemorrhage played any part in the development of the granuloma, and simple surgical blockage of the pathway of ventilation of the pneumatic system appeared to be the only initiating factor. One possible mechanism is that tissues which become bathed with oedematous fluid tend to undergo degenerative changes which may proceed to fatty degeneration and the liberation of fat and cholesterol crystals into the affected tissues and into the air space cavity (Eggston and Wolff 1947). There was certainly some evidence that this did occur, not only in the lining cells, but also in deeper regions of the mesenchymal tissue. Focal areas showing cytoplasmic and nuclear degenerative changes were often found within the proliferating mesenchymal masses.

In addition, however, it is known that alpha and beta lipoprotein molecules, which contain the major part of the plasma cholesterol, pass through the capillary membrane, and that the lymph acts as the sole means of transfer back to the blood stream for those not utilized by the cells (Yoffey and Courtice 1956). It seems reasonable that, under the conditions of the experiment, they may accumulate in the tissues and in the air space for the same reasons that cause the protein level to rise. In either case, however, the physico-chemical mechanism by which the crystals of cholesterol are deposited is unknown.

(h) <u>The multipotentiality of mesenchymal cells.</u>

The fatty and myeloid changes observed in the proliferating

vascular mesenchyme reflect the great potentiality for differentiation which mesenchymal cells seem to possess.(Ham and Leeson 1961). Foam cells and true fat cells appeared to develop from ordinary mesenchymal connective tissue cells, and these fat cells frequently appeared first in close proximity to the blood vessels ramifying through the tissue. There was no evidence that phagocytosis of fat globules occurred and it was more likely that the fat entered the cells in a soluble form as a function of altered metabolic activity.

The basis of this local change may well have been the alteration in blood flow which followed obstruction of the foramen pneumaticum. Alterations in circulation commonly affect cell activity and alter structure (Johnson 1963) and Clark and Clark (1940) demonstrated that fat shows a tendency to diminish during a period of active circulation, and to increase with the return of "quiet" circulation. They also showed that both an increase and a decrease of fat in the fat cells may occur where no lymphatics are present.

Under the conditions of the experiment where the development of a negative pressure was followed by vascular congestion and dilatation together with the onset of oedema, and where a host of new and often quite large vessels appeared, the circulation through the mesenchymal tissue must have become sluggish. This type of alteration in local haemodynamics leads to the development of a high tissue-fluid protein, and probably also an increased transport of fat from the blood capillaries to the tissue cells. These factors facilitate the development of fatty changes

such as were found in the mesenchymal tissues.

It was of interest that the onset of this change was associated with the awakening in the mesenchymal tissue of haemopoietic activity and the appearance of free myeloid cells. However, as the fatty tissue matured and the vascularity decreased, this activity subsided.

The relative absence of a collagenous intercellular matrix, not only in the cholesterol granulomata, but also in the newly formed tissue as a whole, was also of considerable interest in view of the fact that fibrous changes are commonly found in the pneumatic cells of the human temporal bone following infection. The implication is that the stimulus for proliferation of fibroblasts from the multipotential mesenchymal cell population was lacking in the present study, but is present in infected mastoid cells. It would therefore appear that one of the products of the inflammatory reaction may act as the stimulus, and that this was not operative under the conditions of the present experiment where the air cells were healthy and not infected.

(i) The role of the fine vasculature.

Over a number of years, Trueta (1963) has shown that the endothelial cells of the walls of small blood vessels divide and differentiate into osteoblasts or their precursors. Direct vascular invasion precedes ossification, and bone formation and trabecular orientation are dependent on the pattern and distribution of the fine vasculature.

In the present experiment, two distinct patterns of new bone formation were found in two different areas of the proliferating vascular

mesenchyme. Thedeposition and orientation of the newly-formed bone in these cases was exactly moulded about the vascular pattern in the two areas. Just beneath the lining epithelium where the fine vessels were orientated parallel with the epithelium, flat plates of bone were formed. Deeper within the mesenchymal masses where the new vessels were arranged in a rather tangled meshwork, the new bone was deposited in a complex, reticular pattern.

The osteoblastic activity was probably induced, or at least supported, by the high tissue-fluid protein resulting from the passive hyperaemia of the oedematous tissue as shown by Johnson (1963). However, the mechanism of bone deposition in the centre of the cholesterol granulomata may have been somewhat different. Blood vessels were found around the periphery of the granulomata rather than in its centre where the new bone was first laid down. It may, therefore, be that the mesenchymal cells in this situation were transformed into osteoblastic cells as a result of relative ischaemia and/or the irritative effect of the presence of cholesterol crystals. Infection was not a factor in the new bone deposition, as it often is in the pneumatic system of the human temporal bone under clinical conditions (p. 54).

(j) <u>The degree of pneumatisation.</u>

The lining and the oedematous changes, the mesenchymal proliferation and the fatty and myeloid changes all occurred more rapidly in partially than in fully pneumatised humeri after obstruction of the pneumatic foramen. Three factors appeared to be of importance:-

a) The greater vascularity of the pneumatising bone.

The greater degree of vascularity of the pneumatising bone probably facilitated the fluid transudation by providing a larger filtering surface than was present in fully pneumatised bones. In this regard, it is interesting to recall that the first evidence of tissue oedema and lining cell changes was found in relation to the most highly vascular part of the subepithelial tissue, in other words that area subjacent to the advancing front of the air space.

b) The smaller size of the air space in partially pneumatised bones.

The smaller the size of the air space cavity that is obstructed, the more rapidly is a negative pressure produced following oxygen absorption. Accordingly, it is not surprising that vascular and oedematous changes developd more rapidly in the smaller air-containing systems.

c) The amount of mesenchymal tissue.

When compared with fully pneumatised bones, bones in which air space development is not yet complete have a far greater amount of subepithelial mesenchymal tissue, and far more bone marrow, present. Following obstruction of the foramen pneumaticum, the changes of mesenchymal proliferation and of conversion of mesenchymal into fatty and myeloid cells are likely to be observed at an earlier stage in young bones where the mesenchymal cell population is greatest and has the highest growth potential. This was the case in the present experiment.

However, cholesterol granulomata were more common in fully than in partially pneumatised experimental bones. The reason for this is not

entirely clear but it may be that in the presence of a smaller air space and with the greater vascularity of the pneumatising state, less mesenchymal degeneration occurs so that the deposition of cholesterol crystals is consequently decreased.

(k) The nature of the obliterative method.

In general, no great difference was found between the muscle graft technique and the methyl methacrylate method in relation to the rapidity of onset of the histopathological changes in the obstructed pneumatic system of the humeri. There was a suggestion, however, that the lining cell changes and the mesenchymal proliferative changes occurred a little more rapidly after the muscle graft operation. Probably the products of muscle graft degeneration released into the air space played a part in this, as no such products were released as a consequence of the methyl methacrylate procedure.

3. Pneumatisation arrest.

In the present study, it was observed that the subepithelial tissue deep to the advancing front of the air space was actively hyperaemic because of the enlargement and transition of the simusoidal pattern of the subjacent marrow into a vascular network of considerable size. In association with this change, there occurred a local increase in osteoclastic activity, and the disappearance of fat with a shrinkage of fat cells and their ultimate conversion to smaller, more dense cells. These observations are in accord with the findings of Johnson (1963) that active hyperaemia supports or even induces osteoclastic activity, and of Clark

and Clark (1940) that fat shows a tendency to diminish during a period of active circulation.

The result of these changes was the disappearance of bone trabeculae in advance of the air space, and the shrinkage of the subjacent marrow tissue. The space created by this decrease in bulk was then filled, under normal conditions, with air at atmospheric pressure.

In other words, it appears that although other factors may be involved, two main ones are required for normal pneumatisation to proceed. These are

i) a local alteration in the vascular pattern which facilitates osteoclastic activity and the metabolic conversion of fat cells into smaller and more dense cells, with a consequent decrease in bulk of tissue

ii) the maintainance of an equality of pressure in the shrinking tissue and the expanding air space.

In the present experiment the cause of the local vascular alteration was unknown, but the maintenance of pressure equality was made possible by the proximity of the expanding axillary air sac.

The role of the lining cells has been the subject of many theories and considerable dispute. The present observations have produced no evidence that the flattened lining cells of the bony air space are necessarily derived from active or passive ingrowth of epithelium from the respiratory tract. It is considerably more probable, from the normal appearance of the cells, and their pathological alterations under

the conditions of the experiment, that the cells are of mesodermal origin, derived from the mesenchyme within the bone itself. Such a transformation of mesenchyme into mesothelium has been observed by Lewis (1923) in tissue cultures.

Following surgical obliteration of the foramen pneumaticum, a negative pressure is created in the air space following absorption of oxygen. In other words pressure equality between the previously shrinking tissue and the previously expanding air space is no longer maintained. Pneumatisation must, therefore, cease.

In addition, however, it has been demonstrated that the subepithelial zone becomes oedematous and that the vessels in the zone undergo considerable dilatation and congestion, the passive hyperaemia of oedematous tissue (Johnson 1963). The vascular pattern prerequisite for pneumatisation becomes altered, and pneumatisation not only ceases, but, because of the newly-imposed haemodynamic situation, the process actually becomes reversed as observed in the present investigation.

BIBLIOGRAPHY.

ADAMS, W.S. (1953). "Deafness and peritubal oedema". Proc. 5th Internat. Cong. Oto-rhino-laryng., (Amsterdam) p. 818.

ADAMS, W.S. (1961) From "Discussion on problems of Tympanoplasty" J.L.O., 75, 942.

ALBRECHT, W. (1924). Pneumatisation und konstitution. Z. Hals-usw. Heilk., 10, 51.

ALMOUR, R. (1933). "Evolution of the mastoid tip cell as a cell system separate from the remainder of the mastoid cells." Laryngoscope, 43, 797.

ALMOUR, R. (1933-a) Practical application of Wittmaack's theory of pneumatisation. Ann. Otol., 42, 112.

AMALGAMATED DENTAL Co., Ltd. Technical Bulletins, No. I. "Sevriton Simplified" - Acrylic Filling Material.

ARDRAN, G.M. (1953) in "Modern trends in Diagnostic Radiology". Ed. J. W. McLaren.

ARMSTRONG, H.G., HEIM, J.W. (1937) "The effect of flight on the middle ear". J.A.M.A., 109, 417.

ASCHAN, G. (1948) "Aero-otitis media and aerosinusitis". Acta-Otolaryng., Supp. 69.

BALLANCE, C.A. (1919) Essays on the surgery of the temporal bone. London: Macmillan Co.

BALLENGER, W.L. (1908) "The meato-mastoid operation in chronic mastoiditis". J.A.M.A., 51, 1062.

BARTH, H. (1930) Studien über die Anatomie, Entwicklungsgeschichte und normale Pneumatisation der menschlichen Schläfenbeinschuppe". Zeit. für Hals-Nasen und Ohrenh., 26, 483.

BAST, T.H., ANSON, B.J. (1949) "The temporal bone and the ear" Illinois: Thomas.

BAST, T.H., FORESTER, H.B. (1939) Origin and distribution of air cells in the temporal bone. Arch. Otolaryng., 30, 183.

BEALES, P.H. (1959) "Problem of the mastoid segment after tympanoplasty." J.L.O., 73, 527.

BECK, K. (1914) "Uber Mittelohrveränderungen nach experimenteller LÆsion der knorpeligen tube". Verhandl. d. deutsch. otol. Gesellsch;. 28/29, 67.

BECK, K. (1919) "Uber Mittelohrveränderungen bei experimenteller LÆsion der Tube". Ztschr. f. Ohrenh., 78, 83.

BEER, G. de (1954) "Archaeopteryx Lithographica". London: Brit. Mus. Nat. Hist.

BEHNKE, A.R. (1945) "Physiologic effect of pressure changes with reference to Otolaryngology". Arch. Otolaryng., 42, 110.

BELLAIRS, A. D'A., JENKIN, C.R. (1960) Chapt. 7 in Biology and Comparative Physiology of Birds (Ed. A. J. Marshall). New York & London: Academic Press.

BENNETT, A (1933) "Cure of mastoid fistulae with fat graft". Med. Ann. Dist. Columb., 2, 117.

BERGER, A.J. (1960) Ch. 8 in A. J. Marshall's "Biology and Comparative Physiology of Birds". New York & London: Academic Press.

BEST, C.H., TAYLOR, N.B. (1961) The physiological basis of medical practice. Bailliere, Tindall & Cox, Ltd., London.

BEZOLD, F (1893) "Die Krankheiten des Warzenteiles". Handbuch der Ohrenh., Schwartze, Bd. 2. Leipzig: F. Vogel.

BHADURI, J.L., BISWAS, B., DAS, S.K. (1957) "The arterial system of the domestic pigeon". Anat. Ans., 104, 1.

BIRRELL, J. F. (1956) "Black cellular cholesteatosis in childhood". J.L.O., 70, 260.

BLAKE, C.J. (1906) The value of the blood clot as a primary dressing in mastoid operations. B.M.J., 2, 1387.

BLEGVAD, N, R (1941) "Tubal occlusion or catarrh of the middle ear". Acta Otolaryng., 29, 178.

BLOOM, M.A., DOMM, L.V., MALMANDOV, A.V., and BLOOM, W. (1958). "Medullary bone of laying chickens". Amer. J. Anat., 102, 411.

BLOOM, M.A., McLEAN, F.C., BLOOM, W. (1942) "The formation of medullary bone in male and castrate pigeons under the influence of sex hormones." Anat. Rec., 83, 99.

BLOOM, W., BLOOM, M.A., McLEAN, F.C. (1941) "Medullary bone changes in the reproductive cycle of female pigeons". Anat. Rec., 81, 443.

BLUMSTEIN-JUDINA, B. (1905) "Die Pneumatisation des Markes der Vogelknochen". Anat. Hefte., 29, 1.

BONDY, G. (1910) "Totalaufmeisselung mit Erhaltung von Trommelfell und Gehörknöchelchen". Monatsschr. f. Ohrenheilk, 44, 15.

BRADLEY, O.C. (1960) "The structure of the fowl" - 4th Ed. Oliver and Boyd.

BRAGDON, J.H., FOSTER, L., SOSMAN, M. (1949) "Experimental infarction of bone marrow". Am. J. Path., 25, 709.

BREMER, J.L. (1940) "The pneumatisation of the humerus in the common fowl and the associated activity of Theelin". Anat. Rec., 77, 197.

BROCK, W. (1926) "Trommelfellbild und Pneumatisation des Warzenteiles". Z. Hals-usw. Heilk., 15, 241.

BROOKES, M. (1957) "Femoral growth after occlusion of the principal nutrient canal in day-old rabbits". J. Bone & Joint Surg., 39-B, 563.

BROOKES, M., HARRISON, R.G. (1957) "Vascularization of the rabbit femur and tibiofibula". J. Anat., 91, 61.

BROWN, L.G. (1930) "The triumphs and failurs of the mastoid operation". Proc. Roy. Soc. Med. (Sect. Otol.), 23, 385.

BRYANT, W.S. (1906a) "The radical mastoid operation modified to allow the preservation of normal hearing". Trans. Am. Laryng. Rhin. & Otol. Soc. 10, 292.

BRYANT, W.S. (1906b) in discussion on "The value of the blood clot as a primary dressing in mastoid operations". (Blake) B.M.J. 2, 1390.

BUCH, N.H., JORGENSEN, M.B. (1964) "Eustachian tube and middle ear". Arch. Otolaryng., 79, 472.

BUCK, A.H. (1880) Quoted by Mollison (1930).

BUONOCORE, WILEMAN, and BRUDEVOLD, (1956) Quoted in Tech. Bull. No. 1., Amalg. Dental Co. Ltd.,

BURTON, K. (1955) A study of the conditions and mechanism of the Diphenylamine Reaction for the Colorimetric estimation of Desoxyribonucleic acid. J. Biochem., 62, 315.

CAMPANA, J.C. (1875) "Anatomie de L'appareil pneumatique - pulmonaire, etc., chez le poulet". Paris: Masson.

CAMPBELL, E.H. (1955) "Radical mastoidectomy with primary split thickness skin grafting". Arch. Otolaryng. 61, 151.

CARR, A (1964) The Reptiles. Time - Life International.

CHANG, H.T., MARGARIA, R., GELFAN, S. (1950) "Pressure changes and Barotrauma resulting from decompression and recompression in the middle ear of monkeys". Arch. Otolaryng., 51, 378.

CHARLAND, R.A. (1960) "New method of Tympanoplasty". Laryngoscope 70, 1699.

CHEATLE, A.H. (1910-a) Twenty specimens of chronic middle ear suppuration and its sequelae. Proc. Roy. Soc. Med. (Sect. Otol) 3, 41.

CHEATLE, A.H. (1910-b) The infantile types of the temporal bone and their surgical importance. Lancet, 1, 491.

CLARK, E.R., CLARK, E.L. (1940) Microscopic studies of the new formation of fat in living adult rabbits. Am. J. Anat., 67, 255.

CLAUS, G. (1930) "Experimentelle Studien über den verschluss der Tuba Eustachii beim Hunde". Ztschr. f. Hals-, Nasen-, u. Ohrenh., 26, 143.

DAGGETT, W.I. (1949) "Operative treatment of chronic suppurative otitis media". J.L.O. 63, 635.

DELREKAMP, G. (1906) "Das verhalten der knochenarterien bei knochenerkrankungen und Frakturen". Fortschr. Geb. Röntgen-strahlen, 10, 219.

DIAMANT, M. (1940) "Otitis and Pneumatisation of the Mastoid Bone". Acta Otolaryng., supp. 41.

DICKSON, E.D.D., Mc.GIBBON, J.E.G., and CAMPBELL, A.C.P. (1943). "Acute otitic barotrauma". J.L.O., 58, 465.

DOAN, C.A. (1922) "The capillaries of the bone marrow of the adult pigeon". Johns Hopkins Hospital Bull., 33, 222.

DOAN, C.A. (1922) "The circulation of the bone marrow". Carnegie Contrib. to Embryol., 14, 27.

DODDS, G.S. (1930) "Row formation and other types of arrangement of cartilage cells in endochondral ossification". Anat. Rec., 46, 385.

DODGE, H.W., CRAIG, W.M. (1953) "Acrylic cranioplasty". Proc. Mayo Clinic, 28, 256.

DRINKER, C.K., DRINKER, K.R., LUND, C.C. (1922) "The circulation in the mammalian bone marrow". Am. J. Physiol., 62, 1.

DRINKER, C.K., FIELD, M.E. (1931) The protein content of mammalian lymph and the relation of lymph to tissue fluid. Am. J. Physiol., 97, 32.

DRINKER, C.K., FIELD, M.E., HOMANS, J. (1934) "The experimental production of oedema and elephantiasis as a result of lymphatic obstruction. Am. J. Physiol., 108, 509.

ECKERT-MÖBIUS, A (1938) "Vergleichend anatomische Untersuchungen und Pneumatisationslehre". Acta. Otolaryng., 26, 115.

ECKERT-MÖBIUS, A (1924) Ueber die Rolle der gefabhaltigen knorpelkanale bei der enchondralen Verknöcherung. Deutsch. med. Wochenschr, 50, 1798.

EGGSTON, A.A., WOLFF, D. (1947) "Histopathology of the Ear, Nose & Throat" Baltimore: Williams & Wilkins Co.

FARRIOR, J. (1949) "The Radical mastoidectomy: anatomical considerations in surgical technique". Surg., Gyn., and Obstet. 89, 328.

FELL, H.B. (1925) "The histogenesis of cartilage and bone in the bng bones of the embryonic fowl". J. Morph., 40, 417.

FISHER, H.I. (1955) "Major arteries near the heart in the whooping crane". Condor, 57, 286.

FLISBERG, K., INGELSTEDT, S., ORTEGREN, U. (1963-a)
"Clinical volume determination of the air-filled ear space".
Acta. Otolaryng., Supp. 182, p.39.

FLISBERG, K., INGELSTEDT, S., ORTEGREN, U. (1963-b)
On middle ear pressure. Acta Otolaryng., Supp. 182, p.43.

FLISBERG, K., INGELSTEDT, S., ORTEGREN, U. (1963-c)
"The valve and locking mechanisms of the Eustachian tube".
Acta Otolaryng., Supp. 182, p.57.

FOOTE, J.S. (1921) "The circulatory system in bone".
Smithsonian Misc. Coll., 72, no. 10

FORGET, (1860) L'Union Medicale, May 1st.
Quoted by Stevenson & Guthrie (1949)

FOSTER, L.N., KELLY, R.P., WATTS, W.M. (1951) "Experimental infarction of bone and bone marrow". J.Bone & Joint Surg., 33-a, 396.

FOWLER, Jr., E.P. (1945) "Causes of deafness in Flyers". Arch. Otol, 42, 21.

FRIEDMANN, I. (1955-a) Comparative pathology of otitis media - experimental and human. I. J.L.O., 69, 27.

FRIEDMANN, I. (1955-b) Comparative pathology of otitis media - experimental and human. II. J.L.O., 69, 588.

FRIEDMANN, I. (1957) Pathology of otitis media with particular reference to bone changes. III. J.L.O., 71, 313.

FRIEDMANN, I. (1959) Epidermoid cholesteatoma and cholesterol granuloma, experimental and human. Ann. Otol., 68, 57.

FRIEDMANN, I. (1963) "Pathology of secretory otitis media".
Proc. Roy. Soc. Med., 56, 695.

FRITZ, M.H., CRAWFORD, G.B. (1960) "An evaluation of the Rambo Primary closure of the radical mastoidectomy wound."
Trans. Amer. Acad. Ophth. Otolaryng., 64, 159.

GADOW, H., SELENKA, E. (1891) in H.G. Bronn's "Klassen und Ordnungen des Tierreiches", Bd. 4, Abt. 4, Vögel.
Leipsig: Wintersche Verlagsbuchhandlung.

GLENNY, F.H. (1955) Modifications of pattern in the aortic arch system of birds and the phylogenetic significance. Proc. United States Nat. Mus., 104, 525.

GORNALL, A.G., BARDAWILL, C.J., DAVID. M.M., (1949) Determination of serum proteins by means of the Biuret reaction. J. Biol. Chem., 177, 751.

GRAHNE, B. (1963) "Cavity filling technique in mastoid surgery". Acta. Otolaryng. 56, 437.

GRASSE, PIERRE-P. (1950) "Traite de Zoologie". Vol. 15, Oiseaux. Paris: Masson.

GRAY, H. (1930) Anatomy of the Human Body. Edn. 22., Ed. W. H. Lewis. Philadelphia: Lea & Febiger.

GREVEN, H. (1955) "Tierexperimentelle Untersuchungen zum Pneumatisationsproblem". Z. Hals-, Nas-, u Ohrenheilk., 167, 590.

GRIPPAUDO, M. (1959) Il granuloma da colesterolo. Valsalva, 35, 160.

GRZIMEK, B. (1933) "Das arteriensystem des Halses und Kopfes, der Vorder - und Hintergliedmabe von Gallus domesticus". Vet. med. Diss., Berlin.

GUILFORD, F.R. (1961) "Obliteration of the cavity and reconstruction of the auditory canal in temporal bone surgery". Trans. Amer. Acad. Ophth. Otolaryng., 65, 114.

GUILFORD, F.R., WRIGHT, W.K. (1954) "Secondary skin grafting in fenestration and mastoid cavities". Laryngoscope, 64, 626.

GUILFORD, F.R., WRIGHT, W.K., DRAPER, W.L. (1958) "Controlled healing of mastoid and fenestration cavities." Trans. Amer. Acad. Ophth. Otolaryng., 62, 455.

GUNDERSEN, T. (1961) "The use of the post-auricular flap in mastoid surgery". Acta Otolaryng., 53, 45.

GUTHRIE, D. (1928) in discussion on "Use of temporal muscle grafts in mastoid operations" (Kisch). J.L.O. 43, 876.

GUTHRIE, D. (1940) The history of otology. J.L.O., 55, 473.

HAINES, R.W. (1933-4) "Cartilage canals". J. Anat., 68, 45.

HAINES, R.W. (1942) "The evolution of Epiphyses and of Endochondral bone" Biol. Rev., 17, 267.

HAINES, R.W., MOHUIDDIN, A. (1962) "Epiphyseal growth and union in the pigeon". J. Fac. Med. Baghdad, Vol 4(N.S.), No. 1, p.4.

HAM, A.W., LEESON, T.S. (1961) Histology, 4th Ed. London: Pitman.

HAMMAR, J.A. (1902) "Studien über die Entwicklung des Vorderdarms und einiger angrenzenden Organe". Arch. für mikros. Anat, 59. Quoted by Diamant (1940).

HAMMARSTEN, O. (1904) A textbook of physiological chemistry. Trans. J. A. Mendel. 4th Ed. J. Wiley & Sons, New York.

HARMA, R., KOSKINEN, O. (1965) "Mastoid osteoplasty with anorganic bone". Acta Otolaryng., 59, 81.

HARTMANN, A. (1879) "Uber Sclerose des Warzenfortsatzes". Z. für Ohrenh., 8, 18.

HAYMANN, L. (1912) "Experimentelle studien zur Pathologie der akutentzündlichen Prozesse im Mittelohr". Arch. f. Ohrenh., 89, 267.

HEADLEY, F.W. (1893) "The air sacs and hollow bones of birds". Nat. Science, 3, 346.

HEATH, C.J. (1906) The cure of chronic suppuration of the middle ear without removal of the drum or ossicles or the loss of hearing. Lancet, 2, 353.

HEINE, B. (1904) "Operationen am Ohr". Berlin: S. Karger.

HERRMANN, R., RIEHM, J. (1961) "Course of the sigmoid sinus and pneumatisation of the mastoid. Also a contribution to the question of secondary sclerosis of the mastoid". H.N.O. (Berl.), 9, 129.

HIMALSTEIN, M.R. (1959) Mastoid pneumatisation. Laryngoscope, 69, 561.

HINTON, J. (1874) The questions of aural surgery. London: King.

HOLLINGER, J. (1908) In discussion on "The meato-mastoid operation in chronic mastoiditis" (Ballenger). J.A.M.A., 51, 1062.

HOLMGREN, G. (1934) "Recherches experimentales sur les fonctions de la trompe d'Eustache". Acta Otolaryng., 20, 381.

HOLMGREN, L. (1940) "Experimental tubal occlusion". Acta Otolaryng., 28, 587.

HOMANS, J., DRINKER, C.K., FIELD, M. (1934) Elephantiasis and the clinical implications of its experimental reproduction in animals. Ann. Surg., 100, 812.

HOUSE, H.P. (1949) "Surgery of the chronically discharging ear". Arch. Otolaryng., 49, 135.

HUDSON, G.E., LANZILLOTTI, P.J. (1955) "Gross anatomy of the wing muscles in the family Corvidae." Am. Midland Naturalist, 53, 1.

HURRELL, D.J. (1934-5) "The vascularization of cartilage". J. Anat., 69, 47.

HUSIK, D.N. (1932) Sclerotic Mastoiditis and Intracranial complications. Laryngoscope, 42 p.519.

HYDE, R.W. (1952) "Aerotitis media". Ann. Otol., 61, 937.

HYNES, W. (1959) "Problem of the mastoid segment after Tympanoplasty". J.L.O. 73, 532.

JACQUEMIN, E. (1842) Sur la pneumaticité des oiseaux. Nova Acta. Acad. Leop. Carol., 19, 285.

JANSEN, A. (1893) "Zur Kenntniss der durch Labyrintheiterung inducirten tiefern extraduralen Abscesse in der hinteren Schaedelgrube". Arch. f. Ohrenh, 35, 290.

JANSEN, A. (1908) in discussion on "The meatomastoid operation in chronic mastoiditis" (Ballenger). J.A.M.A. 51, 1062.

JENKINS, G.J. (1930) in discussion on "The radical mastoid operation". (Stewart & Fraser). Proc. Roy. Soc. Med. (Sect. Otol.), 23, 394.

JOHNSON, L.C. (1963) Morphologic analysis in pathology: The kinetics of disease and general biology of bone. Ch. 29 in "Bone Biodynamics". Henry Ford Hosp. Internat. Symp., Ed. H.M. Frost. Little, Brown & Co., Boston.

JOHNSON, R.W. (1927) "A physiological study of the blood supply of the diaphysis". J. Bone & Joint Surg., 9, 153.

KAUPP, B.F. (1918) Anatomy of the Domestic Fowl. Saunders Co.

KEMBLE, J. (1936). "Hero-Dust". London: Methuen.

KERRISON, P.D. (1930) Diseases of the ear. 4th Ed. Philadelphia & London: Lippincott Co.

KHAN, A (1927) "New method for skin grafting the mastoid cavity". Laryngoscope, 37, 889.

KING, A.S. (1957) "The aerated bones of Gallus domesticus". Acta. Anat., 31, 220.

KING, A.S., PAYNE, D.C. (1962) Maximum capacities of the lungs and air sacs of Gallus domesticus. J. Anat., 96, 495.

KISCH, H. (1928) "Use of temporal muscle grafts in mastoid operations". J.L.O. 43, 856.

KISTLER, G.M. (1934) "Sequences of experimental infarction of the femur in rabbits." Arch. Surg., 29, 589.

KOPETZKY, S.J. (1938) Surgery of the Ear. New York & Edinburgh: Nelson & Sons.

KORNER, O. (1899) "Die eitrigen Erkrankungen des Schläfenbeins". Weisbaden: Bergmann.

KRAINZ W. (1924) "Uber die Auskleidung der Lufthaltigen Warzenzellen". Zeitschr. f. Hals-, Nasen- u. Ohrenheilk., 8, 46.

KUSTER, E. (1889) "Uber die Grundsätze der Behandlung von Eiterungen in starrwandigen Höhlen, mit besonderer Berücksichtigung des Empyems der Pleura". Deutsche med. Wchnschr. 15, 254.

KYES, P., POTTER, T.S. (1934) "Physiological marrow ossification in female pigeons". Anat. Rec., 60, 377.

LANDAUER, W., PFEIFFER, C.A., GARDNER, W., and SHAW, J.C. (1941) "Blood serum and skeletal changes in two breeds of ducks receiving oestrogens". Endocrin. 28, 458.

LANGER, K. (1876) "Uber das Gefässsystem der Röhrenknochen". Denkschr. Kais. Akad. Wiss., 36, 1.

LATIMER, H.B. (1927) "Postnatal growth of the chicken skeleton". Amer. J. Anat., 40, 1.

LAUROWITSCH, Z. (1913) "Zur Technik des Tubenverschlusses mit der Hornbolzen methode". Verhandl. d. deutsch otol. Gesellsch., 22, 273.

LEMOINE, A. (1957) "Vascular changes after interference with the blood flow of the femoral head of the rabbit". J. Bone & Joint Surg., 39-B, 763.

LEMPERT, J. (1949) "Lempert endaural subcortical mastoido-tympanectomy for the cure of chronic persistent suppurative otitis media". Arch. Otolaryng. 49, 20.

LEWIS, W.H. (1923) Mesenchyme and mesothelium. J. Expt. Med., 38, 257.

LEXER, E. (1903) "Die Entstehung entzündlicher Knochenherde und ihre Beziehung zu den Arterienverzweigungen der Knochen". Arch. Klin. Chir., 71, 1.

LEXER, E. (1904) "Weitere Untersuchungen über Knochenarterien und ihre Bedeutung für krankhafte Vorgänge". Arch. Klin. Chir., 73, 481.

LEXER, E., KULIGA, P., TURK, W. (1904) "Untersuchungen über knochenarterien mittelst Röntgenaufnahmen injizierter knochen und ihre Bedeutung für einzelne pathologische Vorgänge am knochen-systeme". Berlin: A. Hirschwald.

LINDSAY, J.R. (1940) Petrous pyramid of the temporal bone. Arch. Otolaryng., 31, 231.

LOCY, W.A., LARSELL, O. (1916) "The embryology of the Bird's lung". Am. J. Anat., 20, 1.

LOEBELL, H. (1937) "Die funktionelle Architektur des Warzen fortsatzes". Acta. Otolaryng., 25, 240.

LUBOSCH, W. (1924) "Die Bildung des Mark knochens beim Hühnchen und bei Säugetieren". Morph. Jb., 53, 49.

MAHONEY, J.L. (1962) "Tympanoacryloplasty". Arch. Otolaryng., 75, 519.

MANASSE, P. (1917) In Ohrenheilk. Gegenw. vol. 9, Handbuch der Pathologischen Anatomie des Menschlichen Ohres, p. 51, Wiesbaden.

MARMEFFE, R. de (1951) "Recherches morphologiques et experimentales sur la vascularisation osseuse". Brussels: Editions Acta. Med. Belg.

MARSHALL, A.J. (1960) "Biology and Comparative Physiology of Birds". New York & London: Academic Press.

MATSUMURA, H. (1955) "Studies on the composition of air in the tympanic cavity". Arch. Otolaryng., 61, 220.

McAULEY, G.O. (1958) The blood supply of the rat's femur in relation to repair of cortical defects. J. Anat., 92, 655.

McGUCKIN, F. (1964) Personal communication.

McLEAN, J.W., KRAMER, I.R.H. (1952) A clinical & pathological evaluation of a sulphinic acid activated resin for use in restorative dentistry. Brit. Dental J., 93, 255.

McMURRICH, J.P. (1923) "The development of the Human Body". Philadelphia: P. Blakeston's Son & Co.

MERIFIELD, D.O. (1963) "Obliteration of the mastoid segment: a clinical review and pilot study of various transplant materials". Ann. Otol., 72, 157.

MEYER, M. (1931-a) "Uber Konstitution und Mittelohrschleimhaut". Z. Hals-usw. Heilk. 29, 106.

MEYER, M. (1931-b) "Uber die entzündlichen Erkrankungen des Mittelohres". Z. laryngol. Rhinol., 20, 89.

MILL, W.A. (1930) "Three cases of conservative mastoid operation with temporal muscle graft". Proc. Roy. Soc. Med. (Sect. Otol.), 23, 401.

MOLLISON, W.M. (1930) A brief survey of the history of the mastoid operation. Proc. Roy. Soc. Med. (Sect. Otol.), 23, 381.

MORGAN, J.D. (1959) "Blood supply of growing rabbit's tibia". J. Bone & Joint Surg., 41-B, 185.

MOSHER, H.P. (1911) "A method of filling the excavated mastoid with a flap from the back of the ear". Laryngoscope 21, 1158.

MOURET, J. (1913) "Etude sur la structure de la mastoide et sur le developpement des cellules mastoidiennes". Annales de maladies de L'oveille, du larynx, du nez et du pharynx, 39.
Quoted by Diamant (1940).

MUTERMAN, Y., OJALA, L. (1949) "Primary reduction of a large operation cavity in Radical mastoidectomy with a muscle-periosteal flap". Acta. Otolaryng., 37, 245.

MULLER, B. (1908) "The air-sacs of the pigeon". Smithsonian Misc. Coll., 50, 364.

MURAKAMI, M., KUDO, S. (1957) "Stenosis of the Eustachian tube and pneumatisation of the temporal bone". Hirosaki Med. J., 8, 636.

NEUGEBAUER, L.A. (1845) "Systema venosum avium". Nova Acta Acad. Nat. Curiosorum, Tom 21., Supp., 517.

NISHIMURA, I (1936) "Roentgenological and histological study on mechanism of occurrence and development of pneumatisation in temporal bone". Ausz. z. Otol. usw. Tokyo, 40.
Quoted by Diamant (1940).

OJALA, L. (1950). "Contribution to the physiology and pathology of mastoid air cell formation". Acta. Otolaryng., Supp. 86.

OJALA, L. (1953) "Pathogenesis and histopathology of chronic adhesive otitis. Arch. Otolaryng., 57, 378.

OJALA, L. (1957) "Pneumatisation of the bone and environmental factors - experimental studies on chick humerus". Acta. Otolaryng., Supp. 133.

OPHEIM, O. (1941) Chronic middle ear inflammation and the pneumatic cellular system in the mastoid process. Acta Otolaryng., 29, 56.

OPHEIM, O. (1944) "The pneumatic conditions of the human temporal bone in the light of experimental researches on the development of the air space in the chick humerus". Acta Otolaryng., Supp. 54.

OTTE, W. (1928) "Die Krankheiten des Geflügels mit besonderer Berücksichtigung der Anatomie und der Hygiene". Berlin: Schoetz.

PAGENSTECHER, H. (1958) "Non-pneumatised mastoid bone in deformities of the middle ear". H.N.O. (Berl.), 7, 109.

PALVA, T. (1963) "Surgery of chronic ear without cavity". Arch. Otolaryng., 77, 570.

PAUSE, R. (1893) "Stackes Operationsmethode zur Freilegung der Mittelohrraeume waehrend der ersten Jahres ihrer Anwendung in des Ohrenklinik zu Halle". Arch. f. Ohrenh. 34, 280.

PECK, D. (1961) "Musculoplasty and Temporal Bone Procedures." Arch. Otolaryng., 74, 677.

PEER, L. A. (1943) "Diced cartilage grafts". Arch. Otolaryng., 38, 156.

PEER, L.A. (1955) "Transplantation of tissues" - 2 vols. Baltimore: Williams and Wilkins Co.

PETIT, J.L. (1774) "Traite des maladies chirurgicales". Paris.

PFEIFFER, C.A., GARDNER, W. (1938) "Skeletal changes and blood serum calcium level in pigeons receiving oestrogens". Endocrin., 23, 485.

PFEIFFER, C.A., KIRSCHBAUM, A., GARDNER, W. (1940) "Relation of oestrogen to ossification and the levels of serum calcium and lipoid in the English sparrow, Passer Domesticus". Yale J. Biol. & Med., 13, 279.

POLITZER, A. (1883) Diseases of the Ear and adjacent organs. (English Translation: J.P. Cassells). London: Bailliere, Tindall and Cox.

PORTMANN, A. (1950) in P-P Grasse's "Traite de Zoologie," Vol. 15, Oiseaux, p. 245-249. Paris: Masson.

PRATT, C.W.M. (1961) "Effect of age on the arrangement of fibres in the bone matrix of the femur of the domestic fowl". J. Anat., 95, 110.

PRATT, C.W.M., McCANCE, R.A. (1960) "Severe undernutrition in growing and adult animals" Brit. J. Nut., 14, 75.

PROETZ, A. (1922) "Observations upon the formation and function of the accessory nasal sinuses and the mastoid cells". Ann. Otol., 31, 1083.

QUAIN, J. (1915) "Elements of Anatomy". Ed. E. Schafer, J. Symington, T. H. Bryce. London: Longmans, Green & Co.

RAHM, W.E., STROTHER, W.F., LUCCHINA, G., and GULICK, W.L. (1958). "Effects of air pressure on the ear". Ann Otol., 67, 170.

RAINER, A. (1938) "Development and construction of pyramidal cells". Arch. Ohren- Nasen- u. Kehlkopfh., 145, 3.

RAMBO, J.H.T. (1958) "Musculoplasty: a new operation for suppurative middle ear deafness". Trans. Amer. Acad. Opth. Otolaryng., 62, 166.

REICHEL, S.M., (1947) "Vascular system of the long bones of the rat". Surgery, 22, 146.

REIK, H.O. (1906) The blood-clot dressing in mastoidectomy, considered physiologically. J.A.M.A. 46, 935.

REWELL, R.E. (1963) Pathology of the upper respiratory tract. Livingstone, Ltd, Edinburgh & London.

RIDDER, O., RAUCH, V.M., SMITH, G.C. (1944) "Changes in medullary bone during the reproductive cycle of female pigeons". Anat. Rec., 90, 295.

RODRIGUEZ, L. (1952) "Injertos de grasa en la Radical de ordo". Revista espanola oteneurooftalmelogia, 11, 191.

ROUVIERE, Mme H. (1910) Contribution a l'etude du developpement de l'antre mastoidien et des cellules mastoidiennes chez l'homme. These, Montpellier. Quoted by Diamant (1940).

RUBASCHEWA, A., PRIWES, M.G. (1932) "Blutversorgung der langen Röhrenknochen des Hundes". Ztschr. Anat., 98, 361.

RUEDI, L. (1937) "Die Mittelohrraumentwicklung vom 5. Embryonal-monat bis zum 10. Lebensjahr". Acta Otolaryng., Supp. 22.

SALA, O., De'STEFANI, G. (1963) "Modifications caused by the occlusion of the tube on the mucosa of the middle ear". Laryngoscope, 73, 320.

SAPPEY, C. (1847) "Recherches sur L'Appareil des Oiseaux" Paris: Bailliere.

SAPY, B. (1941) "Uber das arteriensystem der Hausvögel" Vet. med. Diss., Budapest.

SCHILLER, A. (1963) "Mastoid osteoplasty". Arch. Otolaryng., 77, 475.

SCHWARTZE, H.H., EYSELL, C.G. (1873) Uber die Künstliche Eröffnung des warzen fortsatze. Arch. f. Ohrenh., 7, 157.

SCHWARZ, M. (1929) "Die Bedeutung der hereditären Anlage für die pneumatisation der Warzenfortsätze und der Nasennebenhöhlen". Arch. für Ohren- Nasen- und Heilk., 123, 161.

SCHWARZBART, A. (1958) "A reappraisal of the clinical and morphological classification of the tympanic spaces and Eustachian tube". Ann. Otol., 67, 241.

SCHWARZBART, A. (1959) "Pneumatisation of the temporal bone: a new concept." J.L.O., 73, 45.

SCHWARZBART, A. (1960) "Repneumatisation of the tympanic cavity in Otosurgery". J.L.O., 74, 541.

SELENKA, E. (1866) "Beitrag zur Entwicklungsgeschichte der Luftsäcke des Huhns". Zeitschr. f. Wissen. Zool., 6, 178.

SENTURIA, B.H., CARR, C.D., AHLVIN, R.C. (1962) Middle ear effusions: Pathologic changes of the mucoperiosteum in the Experimental animal. Ann. Otol., 71, 632.

SENTURIA, B.H., GESSERT, C.F., CARR, C.D., and BAUMANN, E.S. (1961) "Aerotitis media - a comparison of Barotraumatic effusions with middle ear fluids of nonbarotraumatic origin". Arch. Otol., 74, 141.

SHAMBAUGH, G.E., Jr. (1959) Surgery of the Ear. Philadelphia & London: W.B. Saunders Co.

SIEBENMANN, F. (1893) "Die Radical-operation des Cholesteatoms mittelst Anlegung breiter permanenter Oeffnungen gleichzeitig gegen den Gehörgang und gegen die retroauriculare Region". Berl. Klin. Wchnschr, 30, 12.

SILBEGER, H. (1950) "Uber das Ausmass des Masteidpneumatisation beim Menschen". Acta. anat. (Basel)., 11, 215.

SIDONETTA, B. (1949) Chronic cholesteatomatous and chronic cholesterinic otitis. Acta Otolaryng., 37, 509.

SIMONS, J.R. (1960) in A.J. Marshall's "Biology and Comparative Physiology of Birds". New York and London: Academic Press.

SINGLETON, J.D. (1944) Pneumatisation of the adult temporal bone; the mastoid portion. Laryngoscope, 54, 324.

SIRAUD, M. (1895) "Recherches anatomiques sur les arteres des os longs". Paris: Dain, O.

SISSON, S., GROSSMAN, J.D. (1953) "The Anatomy of the Domestic Animals" - 4th Ed. Philadelphia & London: Saunders Co.

SMITH, L.C. (1964) Personal communication. (Amalgamated Dental Co., Ltd.).

SONNESCHEIN, R. (1936) A brief consideration of the history of the development of mastoidectomy. Surg., Gynaec. & Obstets., 62, 523.

SPENCE, W.T. (1954) "Form-fitting plastic cranioplasty". J. Neurosurg., 11, 219.

STACKE, L. (1889) Berl. Klin. Wchnschr., 26, 350.

STACKE, L. (1893) "Stacke's Operationsmethode" Arch. f. Ohrenh. 35, 145.

STEVENSON, R.S., and GUTHRIE, D. (1949) A history of Otolaryngology. Edinburgh: E. & S. Livingstone Ltd.

STEWART, J.P. (1928) The histopathology of mastoiditis. J.L.O., 43, 689.

STEWART, J.P., FRASER, J.S. (1930) "The Radical Mastoid operation" Proc. Roy. Soc. Med. (Sect. Otol.) 23, 390.

STRAATSMA, C., PEER. L. (1932) "Repair of postauricular fistula by means of free fat grafts". Arch. Otolaryng., 15, 620.

STRASSER, H. (1877) "Uber der Luftsacke der Vogel". Morph. Jahrb., 3, 179.

STRESEMANN, E., (1927-1932) Handbuch der Zoologie. Vol. 72 Aves.

STUMP, C.W. (1925) The histogenesis of bone. J. Anat., 59, 136.

SUAREZ, C. (1949) "Radical de oido con injeerto de grase libre". Revista clinica espanola, Madrid, 34, 483.

TAMARI, M.J., SZANTO, P.B. (1954) Morphologic changes in the mastoid bone under antibiotic therapy. Arch. Otolaryng., 60, 133.

TAYLOR, T.G., MOORE, J.H. (1953) "Avian medullary bone" Nature, 172, 504.

THOMPSON, E., HOWE, H.A., HUGHSON, W. (1934) "Middle ear pressure and auditory activity". Am J. Physiol., 110, 312.

THOMSON, J.A. (1923) The Biology of Birds. London: Sidgwick & Jackson Ltd.

THORBURN, I.B. (1960) "A critical review of Tympanoplastic surgery". J.L.O., 74, 447.

THORBURN, I.B. (1961) "Experience with Pedicled Temporal muscle flaps in radical mastoid and tympanoplasty operations" J.L.O., 75, 885.

TIEFFENBERG, D. (1949) "Centribucion al tratamiento quirurgico de la otorrea cronica colesteatomatosa. Antrotomia con relleno oseeo". Rev. Argent. Otorinolaring., 18, 283.

TIEFFENBERG, D. (1964) comment on "mastoid osteoplasty" (Schiller 1963) Arch. Otolaryng., 79, 540.

TOYNBEE, J. (1860) "The diseases of the ear". London: Churchill.

TRUETA, J. (1957) "The normal vascular anatomy of the human femoral head during growth". J. Bone & Joint Surg., 39-B, 358.

TRUETA, J. (1963) The role of the vessels in osteogenesis. J. Bone & Joint Surg., 45-B, 402.

TRUETA, J., BARCLAY, A.E., DANIEL, P.M., FRANKLIN, K.J., and PRITCHARD, M.M.L. (1947) "Studies of the renal circulation". Oxford: Blackwell.

TRUETA, J., CAVADIAS, A.X. (1964) "A study of the blood supply of the long bones". Surg., Gyn., Obstet., 118, 485.

TRUETA, J., HARRISON, M.H.M. (1953 "The normal vascular anatomy of the femoral head in adult man". J. Bone & Joint Surg., 35-B, 442.

TUCKER, E.J. (1956) "Studies of the use of cultured calf bone in human bone grafts". Clin. Orthopaedics, 7, 171.

TUMARKIN, A. (1957) "On the nature and vicissitudes of the accessory air spaces of the middle ear" - six parts. 71, 65-99, 137-161, 211-248.

TUMARKIN, A. (1959) On the nature and significance of hypocellularity of the mastoid. J.L.O., 73, 34.

TURNBULL, L. (1862) Med. Surg. Rep. Philadelph. Feb. 15th, 22. Quoted by von Troltsch (1873).

URIST, M.R., DEUTSCH, N.M. (1960) "Osteoporosis in the laying hen". Endocrin., 66, 377.

VAN DEINSE, J.B., VAN DEN BORG, R.E. (1958) "Attico-antrotomy with abdominal fat transplantation and myringoplasty" Pract. Otorhinolaryng. (Basel), 20, 327.

VAN DISHOECK, H.A.E. (1941) "Negative pressure and loss of hearing in tubal catarrh". Acta. Otolaryng., 29, 303.

VAN TYNE, J., BERGER, A.J. (1959) "Fundamentals of Ornithology". New York: J. Wiley & Sons, Inc.

VICENCIO, A.B. (1956) A retroauricular pedicle skin graft to line the radical mastoid cavity. Arch. Otolaryng., 63, 296.

VON BERGMANN, E. (1889) "Die chirurgische Behandlung von Hirnkrankheiten". Berlin.

VON TROLTSCH, A. (1861) "Ein Fall von Anbohrung des Warzenfortsatzes bei Otitis interna mit Bemerkungen über diese operation". Virchow's Archiv. 21, 295.

VON TROLTSCH, A. (1873) "Lehrbuch der Ohrenheilkunde mit Einschluss der Anatomie des Ohres". Leipzig: Vogel.

WALLACE, G.J. (1963) "An introduction to Ornithology". New York: Macmillan Co.

WALSH, T.E. (1958) from discussion on "Controlled healing of mastoid and fenestration cavities" (Guilford, Wright, Draper). Trans. Amer., Acad. Ophth., Otolaryng., 62, 464.

WELLER, W.A. (1958) "Fibrosing Mastoiditis". Ann. Otol., 67, 112.

WELTY, C.F. (1908) in discussion on "The meato-mastoid operation in chronic mastoiditis" (Ballenger). J.A.M.A. 51, 1062.

WESTPFAHL, U. (1960-61) "Das Arteriensystem des Haushuhnes (Gallus domesticus)." Wiss. Zeits. Humboldt., 10, 93.

WHISTON, G.C. (1940) "A histological study of the growing avian femur (Gallus domesticus) following experimental dislocation of the hip". Anat., Rec., 76, 499.

WHITING, F. (1905) "The Modern Mastoid operation" London: Rebman Ltd.,

WILDE, W. (1853) "Aural Surgery". Dublin.

WILDERMUTH, H.A. (1877) "Der feinere Bau der lufthaltigen Vogelknochen nebst Beiträgen zur Kenntnib ihrer Entwicklung". Jena. Zeit. f. Naturwissen., 11, 537.

WILLMER, E.N. (1960) Cytology & Evolution. Academic Press; New York & London.

WIRTH, E. (1935) Experimentelle untersuchungen zur Bakteriologie und Pathologie der chronischen Mittelohrentzündungen. Z. Hals-Nasen- Ohrenh., 37, 316.

WITCHER, J.E., STREIT, A.J. (1963) "Musculoplasty and Musculo-tympanoplasty". Laryngoscope, 73, 185.

WITTMAACK, K. (1918) "Uber die normale und die pathologische pneumatisation des Schläfenbeines". Jena.

WOLBACH, S.B., HEGSTED, D.M. (1952) "Endochondral bone growth in the chick" A.M.A. Arch. Path., 54, 1.

YOFFEY, J.M., COURTICE, F.C. (1956) Lymphatics, Lymph and Lymphoid tissue. E. Arnold, London.

ZAUFAL, E. (1890) "Technik der Trepanation des Proc. Mastoid. nach Köster'schen Grundsätzen". Arch. f. Ohrenh, 30, 291.