THE FORMATION OF BONE IN A CALCIFIED EPI- THELIOMA OF THE SKIN, WITH SOME REMARKS ON METAPLASIA.¹

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(PLATES XXIV.—XXV.)

A NEOPLASM of the skin, which has recently passed through my hands, was found to have undergone ossification. As the tumour is a rare one, and the process by which the bone arises is of great interest, I take this opportunity of describing the specimen, and of setting forward a possible explanation of the manner in which the ossification has taken place. The formation of bone in osteogenetic tumours calls for no explanation; but there are other cases, of which this is an example, in which this tissue arises by a secondary process which appears always to follow necrosis and calcification of the original tumour. Indeed, on taking a wider outlook, we find that bone formation may follow these changes at any site in the body. Numerous examples of this will be quoted later. The ossification in these instances is usually said to be the result of metaplasia, a phenomenon about which varying views are held. One reads, for instance, that metaplasia is a constant physiological process, the conversion of cartilage into bone, and of connective tissue into fat, being given as examples. The latter is nothing but a storing inside the cells of a substance of the nature of paraplasm. The former (except in the unusual cases mentioned below) is a substitution of one tissue by another, the cells of the cartilage taking no active part in the process. It is not, in any sense of the word, a metaplasia of the cells of the cartilage into bone: whether it is one of the cells of the periosteum and marrow into this tissue is a different question.

Metaplasia may be defined as the assumption by one differentiated tissue of the morphological structure and functions of another differentiated tissue. It should be clearly distinguished from the differentiation of embryonic tissues. It is usually found, apart from neoplasms, in association with irritation, mostly of a chronic nature. Metaplasia is direct or indirect: the former, when the fully differentiated cells of the

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part acquire the new characters; and the latter, if they first revert, by a series of divisions, to the degree of differentiation they had reached at some previous stage of their development. A moment's consideration will show that, in the case of a highly differentiated tissue which possesses well-marked anatomical characters, such as an epithelium, metaplasia must be indirect. Let us take as an example a squamous epithelium, such as that of the œsophagus, whose development has been worked out by Schridde (1907²³) in a very able monograph. The only cells that divide and give rise to all its layers are the germinal cells of the rete mucosum. These are the least differentiated; the others acquire fibres, prickles, and keratohyalin granules by a process of differentiation, and coincidentally lose their power of reproduction. But a germinal cell of the œsophagus, under normal conditions, never gives rise to columnar ciliated cells; its own differentiation is such that it can only produce squamous descendants. In order that the former may appear, the germinal cells must revert to the lowly stage of differentiation they had reached at about the end of the fourth month of foetal life, when the œsophagus ceased to be lined by ciliated epithelium. This they do by continued division, a process which is associated with de-differentiation and rejuvenescence (Child, 1915³). The tissue thus regains corresponding potentialities for development; it will be able to produce columnar ciliated as well as, or instead of, squamous cells. This process, which is the expression of a profound metamorphosis of the cells concerned in it, does not, however, extend beyond the potentialities of those from which the tissue has originally been laid down. There is no sound evidence for the view of Lubarsch (1901¹⁴), who admits a metaplasia of connective tissue into epithelium.

When we come to consider the connective tissues, we meet at once with the difficulty that their cells rarely possess a characteristic anatomical structure. With the exception of bone we can usually only tell the nature of a tissue by its matrix. Even the bone corpuscles, when compared with the cells of an epithelium, have a very indifferent appearance. When metaplasia occurs in the connective tissues it is therefore direct. The only recorded instance of this change that I know of is the formation of so-called "cartilage bone," in which the cells of the cartilage are directly converted into bone corpuscles. According to Kaufmann (1911¹¹) it has been observed in the ossification of chondromata, in rickets, in the formation of callus, and even plays a part in the normal development of the mandible and the petrous bone. I have seen analogous appearances in the wall of a large simple cyst of the humerus. Mr. Strangeways has very kindly demonstrated to me sections of his extensive series of chronic bone and joint lesions. These appear to show that this direct transformation of one connective tissue into another may be of more frequent and wider occurrence than is generally supposed.

As will be seen later, the bone has been formed in the present and in all analogous cases by the activity of young, actively proliferating connective-tissue corpuscles. This is, indeed, so in every instance in which it is found in calcified areas outside the skeleton. The ubiquitous fibroblasts, especially when in a state of active proliferation, have a very slight differentiation, and are not (in appearance at any rate) very far removed from the cells of the mesoblast of the embryo. We have therefore no histological means of gauging their potentialities, and should not jump to the conclusion that, when they develop into bone or cartilage, they do so by a process of metaplasia. They are the cells that, of all those in the body, are chiefly concerned with repair. This process, under ordinary conditions, ends with the production of fibrous tissue. I can, however, see no reason why, with unusual stimuli, they should not be able to produce, I will not say almost every kind of mesoblastic tissue, but, at all events, bone. On the following pages I hope to adduce some evidence in support of this view.

1. DESCRIPTION OF THE TUMOUR.

A subcutaneous tumour of the left pectoral region was excised, together with the skin over it, in a healthy male, æt. 41, at Mr. Bromley's out-patients. It lay above and to the outer side of the nipple, and had been noticed for several years. It had slowly increased in size, but not lately. It was hard, tender, and freely movable over the deep tissues.

NAKED-EYE APPEARANCE.—The strip of skin, which is attached to the tumour, is freely movable over it. Except for a slight amount of thinning over its central part, it is of normal thickness, and is separated from the tumour by a small amount of areolar subcutaneous tissue. The growth itself is surrounded by the subcutaneous fat. Its longest diameter, which is parallel to the surface of the skin, measures 19 mm. Its greatest depth is 15 mm. and its thickness 10 mm. It is kidney-shaped, with numerous slight elevations on the surface, and with a depression on its deep aspect. It is white in colour, of the appearance of chalk, and of a bony hardness. No distinct capsule is to be made out with the naked eye, but the subcutaneous tissue appears to be condensed in places on its surface, especially at the depression. A section was made along the greatest diameter of the tumour. Owing to its hardness considerable force had to be employed in making this section. The cut surface consists of a complicated and irregular spongework of hard white material, the meshes of which are filled with fine strands of a softer darker substance, which communicates with the subcutaneous tissue, and obviously represents the connective-tissue septa of the tumour. The hard substance is, for the most part, arranged in the form of thin lamellæ, but near the surface these swell out into rounded masses, about 1 or 2 mm. in diameter, which form the elevations on the surface. The deep depression on the under side extends through about one-third of its substance, which it incompletely separates into two parts, of which one is about one-third the size of the other. The subcutaneous tissue which fills in this depression is condensed, and in it two small, apparently isolated, hard white nodules can be seen.

TECHNIQUE.—The specimen was decalcified *en masse*. The process was

stopped when it was judged to have proceeded far enough, and before complete decalcification had been reached. Embedding was performed in celloidin. The sections were cut parallel to the longest diameter. No attempt was made at serial sections, but the greater part of the tumour was examined.

MICROSCOPIC APPEARANCE.—With a low power the following facts are to be made out (Plate XXIV. Fig. 1). The *skin* is healthy. The epidermis is thin and is thrown into small irregular papillæ. A few sebaceous and sweat glands and small hair follicles are present. The corium is fibrous and, on its deep surface, passes into the fatty subcutaneous tissue. The *tumour* lies in the latter, which is condensed around it as an incomplete, thin, but dense, fibrous capsule. Irregular septa of a very sclerotic connective tissue extend for short distances into its deeper parts (Plate XXIV. Fig. 1, *c*). In the sections towards one edge of the tumour this is divided in two by a broad septum, which contains a group of sweat glands. Sections taken from nearer the centre show that the two parts of the tumour unite superficially to these glands, which are separated from the corium by a layer of subcutaneous tissue as well. The tumour itself consists of irregular wavy and curled bands of a coagulated necrotic material, shown to be epithelium, the central parts of which stain diffusely with eosin. As the periphery is approached the pink becomes masked by a blue colour, due to diffuse hæmatoxylin staining. It is deepest at the edges of the tumour, where the epithelium comes into contact with living fibrous tissue. In the central parts the epithelial strands are separated from each other by empty spaces, or by intervals of a shreddy granular material, the remains of the stroma, which contains numerous crystals of cholesterolin. It is obvious that all the epithelium and nearly all the connective tissue is dead.

The smaller part of the tumour, which is imperfectly divided off by the triangular notch already described, presents a much more irregular and variegated appearance (Plate XXIV. Fig. 1, *b*). Here the masses of dead epithelium are smaller, more delicate, more broken up, and farther apart. They also take on the diffuse hæmatoxylin stain more deeply than does the rest of the tumour. Here, again, this is most intense at their periphery. The spaces between them are occupied by a connective tissue, which is alive, though generally very dense and fibrous. It shows certain changes presently to be described. Gaps occur on the surface between the pieces of dead epithelium, by means of which the stroma is directly continuous with the capsule and the subcutaneous tissue. At the extreme end or apex of this part the masses of dead tissue are widely separated from each other (Plate XXIV. Fig. 1, *a*). At this spot the subcutaneous tissue and fat enter the tumour, and extend for a considerable distance within its substance (to *b*, Plate XXIV. Fig. 1). Attached to and blending with the epithelium in and near this area there are some irregular branching trabeculæ of bone (drawn in black in Plate XXIV. Fig. 1).

Mention has been made of two apparently isolated nodules in the notch on the surface. Others are found in some of the sections outside various parts of the capsule (Plate XXIV. Fig. 1, *e*). They are all to be regarded merely as out-growths, being connected with the epithelium at other levels.

It is necessary to describe the individual structures that compose the tumour in greater detail.

1. *The masses of necrotic epithelium.*—In the deepest parts every vestige of structure and of outline has been lost. They here present a slightly shreddy, but otherwise almost homogeneous appearance, with a few fine cracks, more or less parallel to their long axes, caused by drying and shrinkage of the dead material. As the periphery is reached the cells are found to be in a much better state of preservation (Plate XXV. Fig. 2). Their outlines are more or less plainly visible. They are separated from each other by narrow spaces, a few of which are even bridged by the remains of intercellular processes, thus

proving that they are prickly cells. With iron hæmatoxylin a network of intraprotoplasmic fibrils, characteristic of squamous epithelium, can be demonstrated within their bodies. The majority of the cells contain a clear oval or rounded central space, which had obviously been occupied by the nucleus during life. The fibrils do not encroach on these spaces. In the best preserved parts an occasional horny pearl, stained intensely with eosin or picric acid, is visible (Plate XXV. Fig. 2). It is usually surrounded by concentric layers of flattened cells. Gram's stain for horn is negative, but, as keratinising living cells only are picked out by this method, it is not surprising that it should have failed in the present instance. Hæmorrhages have taken place into some of the masses (Plate XXIV. Fig. 1, *d*). Here the blood pigment has picked out the shapes of the cells in a very characteristic manner. I must emphasise that in none of the sections were there any living epithelial cells found. The epithelium occurs as solid acini, whose edges are often irregular. Isolated groups and solitary epithelial cells are often found in the remains of the stroma.

We have seen that the masses of dead epithelium represent the areas of calcification. The lime salts are deposited within the cells in the form of fine powdery granules, which are generally most numerous around the central nuclear space (Plate XXV. Fig. 2). In the partially decalcified specimen the outer zones only have retained the calcium salts, the centres of the masses being completely decalcified. It therefore follows that calcification was most intense at their periphery. Here the limits of the epithelium are indicated by a deep blue line (in sections stained with hæmatoxylin). Occasionally, however, this line is separated from the stroma by a narrow colourless zone, consisting of a row of epithelial cells, whose outlines are plainly visible with a high power of the microscope, but which contain no trace of calcium salts (Plate XXV. Fig. 3).

2. *The connective tissue.*—In the deepest parts of the tumour it has become liquefied and has disappeared, so that the epithelial masses are separated from each other by empty spaces. The septa that run in from the capsule consist of a very dense fibrous tissue, arranged in strands. Between these there are cracks and spaces, many of which are occupied by cholesterin crystals. The nuclei are few, narrow, and spindle-shaped, and there are remarkably few blood vessels, all of which are small, with very thick walls. Small scattered areas of necrosis occur throughout the fibrous tissue, and the epithelium is often lined by a few dead connective-tissue fibres, which are characterised by absence of nuclei and by being encrusted with lime salts (Plate XXV. Fig. 2).

At the large gap at the apex of the smaller part of the tumour, and to a lesser degree from other parts of the capsule, there is an invasion of the old fibrous stroma by a cellular granulation tissue with abundant young thin-walled blood vessels. This spreads along the spaces between the old fibres, which disappear. It is most abundant around the epithelium (Plate XXV. Fig. 3). It here consists of spindle-shaped fibroblasts, supported by a reticulum of delicate young fibrils. The blood vessels are usually large, they possess an endothelial lining, outside which the connective tissue is often slightly condensed to form an envelope of two or three layers of fibroblasts. Young capillaries bud out from the vessels. The stroma is occupied by numerous lymphocytes, which are usually closely packed around the vessels. Large cells, with one or more small round nuclei and a large amount of a vacuolated basophil cytoplasm, occur in numbers. They are phagocytic, since many of them are charged with a granular brown pigment, probably the remains of the old hæmorrhages. Around the epithelium numerous large basophil multinucleated giant cells are to be seen. Many of these are flattened and applied to the outline of the epithelium, others are rounded or angular and occupy bays in the dead material, that they have obviously eroded for themselves. The majority of them possess irregular protoplasmic processes that penetrate

the epithelium. They appear to have been formed by confluence or by incomplete division of the phagocytic cells, large numbers of which lie between them. Similar giant cells are found around some of the cholesterol crystals, and others are occupied in removing the insoluble remains of the stroma. The granulation tissue often occurs in the centres of the epithelial masses, where it occupies the old connective-tissue papillæ, as is shown by the concentric arrangement of the epithelial cells around them. In such places it attacks the latter in the same manner as on the surface.

Fat cells make their appearance in the older parts of the granulation tissue. They collect into groups, between which a few fibroblasts, phagocytes, and lymphocytes are to be seen (Plate XXIV. Fig. 1, *a*).

There is no clear evidence of the presence of myelocytes or erythroblasts. These cells are extremely difficult to demonstrate in sections. An occasional megakaryocyte is present (Plate XXV. Fig. 3, *e*). The granulation tissue thus assumes all the characters of marrow.

3. The bone.—This tissue occurs almost exclusively at the apex of the smaller part of the tumour (Plate XXIV. Fig. 1), and is invariably associated with the young granulation tissue. It is found as irregular spicules and trabeculæ, which are applied to the epithelium, and give one the impression of having replaced these and of forming the continuation of their outlines (Plate XXV. Fig. 3, *b*). It first appears as small plates, which coalesce, and may eventually completely surround pieces of epithelium, the remains of whose cells are often enclosed within them. It possesses well-marked lamellæ, and contains numerous corpuscles, which lie in lacunæ. By Schmorl's method it is found to be riddled with canaliculi (Plate XXV. Fig. 4). Typical Haversian systems occur (Plate XXV. Fig. 3, *d*), but these are by no means common. Some parts of the bone are powdered over with lime salts, whereas others present the clear appearance of mature tissue. It is obvious that the spicules differ in age. Some are quite mature, stain a purple colour with hæmatoxylin and eosin, and are covered by a thin layer of slightly condensed fibrous tissue, which forms a periosteum for them. Others consist of younger bone, which stains a bright pink by this method.

There is evidence to show that the bone is laid down in two forms, as reticular and as lamellar bone. The former is represented by a few small areas only (Plate XXV. Fig. 3, *c*), whose surface towards the marrow is irregular, and whose matrix consists of interlacing fibres. In the lamellar form one sees a narrow layer of transparent, hyaline, non-staining tissue, which is applied to the outer surface of the calcified epithelium or to a mature piece of bone. It is bounded, on the side towards the marrow, by a row of fusiform or oval cells, whose long axes are parallel to that of the hyaline tissue, which is obviously a layer of very young bone. If, now, another similar layer is formed on its outer side, the cells will represent a regular row of bone corpuscles, the layers themselves forming two parallel lamellæ.

In whatever form the matrix is laid down, the cells that are concerned in its production are always the fibroblasts of the marrow. They are generally oval in shape, others (even when enclosed on all sides by bony matrix) are narrow and fusiform (Plate XXV. Fig. 4). In the deeper layers of the trabeculæ they become triangular or irregular and their bodies give off processes. By Schmorl's method they can be shown to have acquired delicate processes even before they are surrounded by matrix; these project into canaliculi on its surface (Fig. 4). These fibroblasts nowhere assume the shape of osteoblasts, although they have, of course, taken on their functions. Occasionally a lymphocyte is embedded in the superficial layers of the bony reticulum. This is, apparently, an accident; I have never seen signs of the conversion of such cells into bone corpuscles.

These appearances demonstrate the formation of bone by the granulation tissue and its growth at the expense of the latter. Its spread in this direction

is always slight. It soon acquires a sharp outline and a fibrous periosteum. It never projects more than a very short distance beyond the edges of the epithelium to which it is applied. To understand how the thicker plates of bone are produced, it is necessary to examine their lines of junction with the dead tissue. Here the outline is never sharp. The epithelium is split up into small groups or even into solitary cells, which are either attached to the main body, or are completely surrounded by osseous matrix (Plate XXV. Fig. 4). The bone corpuscles are often directly applied to them, and then are bounded on one side only by a lacuna. Others, that are enclosed on all sides by bone, send out canaliculi that are applied to the epithelium, by means of which they come into direct contact with it. Yet others are naked, and have penetrated into its most superficial layers.

A small amount of absorption of bone by means of giant cells has taken place. These are identical with those found around the epithelium (Plate XXV. Fig. 3).

Ossification also starts in the old connective-tissue papillæ within the epithelial masses, when these have been invaded by granulation tissue. The bone also replaces the necrotic and sclerotic calcified areas of old connective tissue, which are first removed by giant cells. I have observed an old papilla which contained numerous cholesterin crystals. It had been invaded by marrow, and bone had appeared. The latter is advancing around the crystals, one of which is completely embedded within its substance, whereas another is surrounded by it on each side for one-half of its longer diameter.

Small spicules of bone are found under the capsule of other parts of the tumour to which young granulation tissue has penetrated. They are few in number, and extremely small.

The above description can be summarised briefly as follows. A slowly growing and clinically innocent acinous epithelial neoplasm was found in the subcutaneous tissue of the pectoral region of a man æt. 41. It is surrounded by a capsule which is however incomplete, and through which a few small processes of epithelium project. It is squamous in type, prickle cells and intracellular fibres being present. Evidence of keratinisation is found. The whole of the tumour is necrotic. The epithelium is calcified, the calcification being most intense at the periphery of the acini. The greater part of the stroma has liquefied and disappeared. A few of its fibres have calcified. The death of the tumour is the result of deficient blood supply, as a few thick-walled vessels are present. At a date subsequent to its death a part of the tumour has been invaded from the subcutaneous tissue by a vascular granulation tissue of the nature of marrow. This has replaced the old stroma and partially removed the calcified dead epithelium by means of foreign body giant cells. A narrow zone of uncalcified epithelium is often present in immediate contact with the granulation tissue. Where its fibroblasts come in contact with the dead masses they acquire delicate processes and lay down bone with lacunæ, canaliculi, lamellæ, and Haversian canals. The bone does not extend any distance into the tumour, but is limited to the edges of the epithelium, except where it replaces small calcified pieces of the old stroma. Naked corpuscles are found among the dead cells in these situations.

II. CLASSIFICATION AND ORIGIN OF THE TUMOUR.

Descriptions of a number of similar new growths of the skin have been published. I do not propose to enter fully into the literature of the subject, which is set out at length in the papers of Murakami (1911¹⁷) and of Strassberg (1911²⁴). I will briefly mention a few cases that throw light on the structure of these neoplasms.

Eve (1882⁶) describes a calcified lobulated tumour of the skin of the back. It consists of columns of small epithelial cells, the centres of which are occupied by granular masses of a fatty substance and cholesterin. It is probably an adenoma of the sebaceous glands. Several members of the patient's family are said to have possessed similar tumours.

Hutchinson (1890⁹) describes and figures an unencapsuled tumour composed of calcified gland lobules, like those of sebaceous glands. The arteries are small and obliterated. A cellular stroma with giant cells is present. Plates of bone are found in contact with the epithelium. He thinks it undesirable to apply the name of epithelioma to these growths, since their course is very slow and perfectly benign.

Denecke (1893⁴) reports on three cases, which are encapsuled, although some degree of infiltration may be present. Keratinisation is excessive, and always precedes the calcification of the epithelium. The blood vessels are very few in number. Ossification is always limited to the site of the calcified epithelium. Osteoblasts are derivatives of the connective-tissue cells. The structure of the undegenerated parts of his specimens is that of an epithelioma.

Murakami (1911¹⁷) records four encapsuled subcutaneous epitheliomata. There is no invasion of the capsule. Marked endarteritis is always present. Calcification is due to malnutrition of the keratinised epithelium. The probable origin is in the sebaceous glands. There is no ossification in his cases.

Strassberg (1911²⁴), in a paper on the formation of bone in the skin, gives an account of two calcifying epitheliomata.

The greater part of the epithelium of the first of these is dead and calcified. A few of the acini are, however, still lined by healthy cells, with all the features of a squamous-celled carcinoma. Keratinised cell nests are present. There is no capsule, and the stroma is cellular with numerous foreign body giant cells around the dead epithelium. Only one small spicule of bone occurs in connection with the epithelium.

The second case is entirely necrotic and calcified. The capsule is imperfect, and the stroma hyaline and often calcareous. Where the epithelium is surrounded by bone the stroma has assumed all the characters of fibrous marrow. Giant cells are occupied in the removal of the calcified epithelium and connective tissue. The bone first makes its appearance in the lacunæ formed on the surface of the epithelium by the giant cells. There are no signs of keratinisation. The margins of the calcified epithelium are bounded by a deep blue line (when stained with hæmatoxylin), between which and the bone a row of colourless cells is frequently found. Strassberg cannot explain these, but does not think that they are decalcified cells, as there is no proof that tissues within the body can be decalcified.

We have here before us a clearly defined group of subcutaneous tumours, composed of squamous epithelium, which have a marked tendency to necrose and to calcify. This is due to the poverty of the blood supply, a fact of which mention is made by every writer. Some

specimens are encapsuled, others have a slight tendency to infiltrate their immediate surroundings. Clinically, they all run a slow course and are quite innocent.

The present specimen agrees perfectly with the more advanced cases that have been quoted, and corresponds very closely with the second of Strassberg's cases (1911²⁴). It therefore falls naturally into this group, which most authors are agreed in classing with the epitheliomata. The chief dissenting opinion on this point is that of Hutchinson (1890⁹), who, solely on clinical grounds, calls his example an adenoma. But, after all, our classification of tumours into innocent and malignant according to their histological structure is a purely empirical one. New growths which usually run a perfectly benign course may occasionally be extremely malignant, without there being any morphological peculiarities to account for this. The reverse is also true, and applies to this case. A tumour that shows signs of infiltration should be placed among the carcinomata. The specimen under consideration is therefore, in my opinion, a calcifying squamous-celled carcinoma of the subcutaneous tissue.

Calcifying epitheliomata are not confined to the skin. In Erdheim's (1904⁵) series of squamous-celled carcinomata of the duct of the pituitary there is one with extensive necrosis and calcification of the epithelium, which has been, in part, replaced by bone. His illustration of it might have been drawn from the case under discussion. Erdheim states that the tumour described by Walker (1902²⁵) as a primary osteoma of the brain is identical with his own. He had examined Walker's sections.

I have little to say about the origin of my case. The presence of a group of sweat glands in the deeper parts of the capsule suggests that it is derived from a cutaneous cell rest or graft, which had been displaced into the subcutaneous tissue. Such a graft, in order to be able to differentiate into sweat glands as well as into a neoplasm, which, whatever its exact origin may be, is certainly not derived from these, cannot have been displaced later than the fifth month of intra-uterine life, when the glands of the skin have been laid down.

III. ORIGIN AND FORMATION OF THE BONE.

The changes that have occurred in the tumour, and that culminated in the appearance of bone, are of two kinds—regressive and progressive. The regressive changes consist of necrosis and calcification of the tumour: to all intents and purposes they are confined to the epithelium, since practically the whole of the stroma had been liquefied. The progressive changes consist of an invasion of a part of the tumour by a vascular granulation tissue, of decalcification and removal of the epithelium (together with the scanty remains of the old stroma) by means of foreign body giant cells, and of its replacement by bone.

Let us first consider the regressive changes. The whole of the epithelium is dead and has undergone calcification. Now calcification, outside the skeleton in an otherwise healthy individual occurs only in dead and dying tissues. I need only instance the calcification of arterio-sclerotic patches of the intima of the aorta, of the degenerated media of arteries, and of old tuberculous nodules in the lungs and lymph glands. v. Kossa (1901¹³) produced degeneration and calcification of the cells of the liver and kidneys after feeding rabbits with certain poisons. Degenerating tissues, therefore, possess affinities for calcium salts. These do not necessarily depend on the formation of soaps, since a calcifying tissue may show hardly any signs of fatty degeneration. Nor do they depend to an appreciable extent on the phosphoric acid contained in the nuclei, as this is only sufficient to produce a very slight amount of calcification. Recently Katase (1913¹⁰) induced this change in various organs of healthy animals after the injection of concentrated solutions of calcium salts. He arrived at the conclusion that every tissue has a certain tolerance for calcium salts. This is lowered where metabolism is impaired, and the salts are precipitated.

The first of the progressive changes in point of time is the invasion of the tumour by granulation tissue from without. The dead tissues liberated decomposition products which acted as irritants to the subcutaneous tissue, and gave rise to a mild plastic inflammation. This reaction may be assumed to have been most intense where the capsule was defective, a condition which is fulfilled at the apex (Plate XXIV. Fig. 1, *a*). Here there is a large gap caused by a deficiency of the capsule and of the epithelium. In the case of the latter tissue the defect may have become enlarged by the subsequent action of the granulation tissue.

Through this gap the granulation tissue has gained access to the interior. Several large blood vessels are found here as well as some lobules of fat. From this point, as well as from less important ones at other parts of the periphery, the granulation tissue has spread between the epithelium, and has formed a new stroma for rather more than a third of the tumour.

A study of the sections makes it quite clear that this granulation tissue has replaced the remains of the original stroma which had escaped liquefaction, and that the latter has taken no part in its formation. Where its sclerotic fibres are calcified they are being removed by giant cells. These, together with small phagocytic cells, are very numerous around the edges of the epithelium.

The granulation tissue rapidly differentiates into a cellular connective tissue with delicate fibres, together with corpuscles that are oval or fusiform, the latter of which preponderate. The larger blood vessels are wide and thin-walled, and numerous capillaries are found everywhere. Large numbers of lymphocytes are scattered about.

They aggregate to form dense masses around the vessels, where megakaryocytes also make their appearance. Later, the fibroblasts become filled with globules of fat. A typical marrow is thus produced, with the characters of that of the skeleton (Plate XXV. Fig. 3).

It may not be out of place to refer to the conclusions as to the origin of the cells of the marrow that were arrived at by Maximow (1907¹⁵) in the course of his experimental production of bone in the kidneys of rabbits. In doing so I have no wish to enter into the subject, since I have not attempted to draw any conclusions from the present case. According to Maximow, the phagocytes are of the nature of polyblasts or fixed wandering cells (*i.e.*, clasmocytes). The giant cells are derived from these cells. The lymphocytes are brought to the part by the blood. They proliferate within and outside the vessels of the marrow, and eventually give rise to all the myelocytes, the megakaryocytes, and the erythroblasts. He excludes a metastasis of cells from the bone marrow, since they were never found in the circulating blood.

The next progressive process that we have to consider is the method by which the epithelium is decalcified and removed. This is effected in two ways. One of these is intimately connected with the laying down of the bone, and will be discussed later. The other is by means of lacunar absorption by giant cells. These, together with the smaller phagocytes, form a ring around the periphery of the greater part of the epithelium where this is in contact with marrow. The giant cells occupy bays on its surface, that correspond in shape to their outlines. Shreds of dead tissue are found within some of them, and it is clear that their function is to erode and to remove the insoluble material. The extreme edges of the epithelium, that are in immediate contact with these cells, have in many places lost their lime salts (Plate XXV. Fig. 3). Again, I have never found granules of calcium salts within the giant cells, the shreds ingested by them being always decalcified. I am therefore led to believe that the action of these cells is twofold: they dissolve out the lime salts from the dead epithelium, and then digest its remains. We will discuss these two actions separately:

(a) *The removal of the calcium salts.*—The marrow, being a very cellular tissue, has an active metabolism, which depends primarily on its nuclei. Therefore, the more nuclear matter a cell possesses in relation to its total volume, the more active will it be in the exercise of its functions. Since the giant cells contain a very large amount of this substance, they may be assumed to be very active. Since oxidation is rapid in such a tissue, a large amount of CO_2 is given off by it. This is very great in the case of the giant cells. CO_2 is, according to Hofmeister (1910⁸), the great solvent of lime salts within the body. The solution is therefore rapidly effected in the immediate vicinity of the giant cells, which are, in fact, closely applied to the calcified tissue.

(b) *The removal of the epithelium.*—The greater part of the dead epithelium, once it is deprived of its lime salts, is liquefied *in situ* by the action of the marrow. The giant cells play an important part

in this respect, as is shown by the lacunæ many of them occupy. Some of the decalcified remains are insoluble; these probably correspond to the areas of keratinisation. They are surrounded and taken up by the giant cells, and then digested by them. These cells therefore possess both extracellular and intracellular ferments.

This view of the twofold action of the giant cells is strengthened by experiments of Hofmeister (1910⁸), who showed that, when plates of bone are subjected to the action of carbonic acid *in vitro*, the matrix remains intact, the calcium salts only being removed.

The third and most important of the progressive processes is the formation of the bone. In Plate XXIV. Fig. 1 all the pieces of this tissue that are visible with a low magnification are drawn in black. It will be seen that they are almost entirely limited to that part of the tumour that had been the first to be invaded by the granulation tissue. The bone occurs as narrow branching spicules, most of which are applied to the surface of the epithelium. This has, here and there, entirely disappeared, the bone being then bounded on all sides by marrow. The connection between these tissues is an invariable one. Where bone is found embedded in the epithelium, it possesses a central core of marrow.

Bone occurs in our tumour in a reticular and in a lamellar form. The former is represented by a few small areas only (Plate XXV. Fig. 3, *c*), and I have never seen signs of its conversion into the other kind. Sacerdotti and Frattin (1902²¹) found that, after ligation of the renal artery in rabbits, the reticular bone that had been deposited in the kidney was removed by lacunar absorption, its place being taken by lamellar bone. There is no evidence of its ultimate fate in the present case.

The lamellar bone is much more abundant. All the larger spicules consist of it. Its lamellæ are conspicuous, although somewhat irregular (Plate XXV. Fig. 3, *b*), its corpuscles are enclosed in lacunæ and communicate with each other and with their surroundings by means of canaliculi (Plate XXV. Fig. 4), and it is penetrated by an occasional Haversian canal (Plate XXV. Fig. 3, *d*).

There is no fundamental difference in the manner in which these two varieties are produced. Such of the fibroblasts of the marrow that happen to be in contact with the surface of the calcified epithelium are responsible for the formation of the bone. Without altering in form they acquire delicate processes, a fact that was first observed by Poscharissky (1905²⁰). They take on the function of osteoblasts without assuming their form. Typical osteoblasts have, however, been described in other examples of epitheliomata of the skin, such as those of Denecke (1893⁴). Later these cells become smaller and angular in shape; they have then become differentiated into typical bone corpuscles. While these changes are going on in the cells, the collagenous fibres of the marrow assume a

translucent hyaline appearance. By taking up lime salts they become converted directly into the fibres of the bony matrix. This is shown very well in the reticular bone, whose fibres do not end at its edges, but are continued into the marrow as connective-tissue fibres.

I cannot sufficiently emphasise the fact that it is the fibroblasts of the marrow that become directly converted into bone corpuscles, and that they do not differ in any structural detail from those that are found in any other granulation tissue. Wherever calcified tissue, in this case epithelium, is present, this change can occur. We have seen that the lime salts are dissolved out of the dead epithelium chiefly by the action of the giant cells. They quickly diffuse out of their bodies and then come into contact with the fibroblasts. They act on the latter as stimuli, to which these respond by taking on the functions of osteoblasts. They convert the connective-tissue fibres into the groundwork of the osseous matrix, which they combine with the lime salts to form true bone. The available calcium salts are soon used up in this process. The bone therefore never extends on the side of the marrow for more than a very short distance.

I therefore believe that the chief and, as far as I can see, the only requirement for the production of bone is the presence of a concentrated solution of calcium salts. This acts as a stimulus to which every young fibroblast in the body is able to react in this way.

I must show that the formation of bone in dead calcified tissue is of frequent occurrence, and that it is widely distributed throughout the body. As the literature on the subject is bulky, I shall content myself with a few striking instances.

Pollack (1901¹⁸) found bone in old calcified tuberculous foci of the lungs in 17 per cent., and (1901¹⁹) in calcareous lymph glands in over 10 per cent. of a series of consecutive autopsies. Bone was present in thirty-one of the forty-four lymph glands he examined, *i.e.*, in over 70 per cent. Poscharissky (1905²⁰) found it around calcareous foci in the lungs, the heart and its valves, in arteries, lymph glands, the meninges of the brain, the choroid and ciliary body of the eye, in nodules of the liver, in a uterine fibroid, a carcinomatous polypus of the stomach, a cystic tumour of the suprarenal, and in a renal "hypernephroma." Moschowitz (1916¹⁶) found bone twice in five cases of calcified hyaline corpora lutea of the ovary. Klotz (1916¹²) observed spicules of bone closely attached to the fractured ends of calcareous rings in arteries.

The formation of bone is readily induced experimentally in rabbits as well as in other animals. Barth (1896²) introduced a large piece of calcined bone into the peritoneum of a cat, and found microscopic spicules of bone in direct apposition with it six weeks later. Sacerdotti and Frattin (1902²¹), Poscharissky (1905²⁰), and Maximow (1907¹⁵) studied it in the kidneys of rabbits after ligature of the renal artery, and Harvey (1907⁷), who gives complete references to the literature on the occurrence of bone in the circulatory system, saw it in eight out of a total of ten in the aorta of the same animal, at the spot where an irritant had been applied.

These instances are, I think, sufficient to prove that this phenomenon is widespread and by no means uncommon. Ossification always occurred in connection with dead calcified tissue, and was always pre-

ceded by the appearance of granulation tissue, which, in the majority of the cases, possessed all the characters of marrow.

Not only are calcium salts essential for the production of bone in the soft tissues, but they are equally indispensable in the skeleton itself.

This has been clearly demonstrated by Barth (1895¹), who investigated the changes that occur in pieces of bone grafted into defects that had been experimentally produced in the skeleton. Once a piece of bone is completely isolated it dies, however quickly it be returned to its natural surroundings. It is invaded by a granulation tissue formed by proliferation of the marrow of the healthy bone into which the piece has been grafted. This penetrates all its Haversian canals, and forms a marrow for it. Osteoblasts line these spaces. By their action new bone is laid down, which replaces the dead bone of the graft without visible absorption. The young bone simply pushes its way into the old, and is everywhere in the most intimate contact with it. Even the minutest particles of dead bone are made use of. They are enclosed in giant cells formed by the confluence of osteoblasts, and form the nuclei of centres of ossification. Lacunar absorption by foreign body giant cells plays no part in this process, except in modelling the young bone after it has been laid down. Precisely the same appearances are observed if ivory or tooth are used for the graft. If, on the other hand, the piece of bone is decalcified before being grafted, it acts simply as a foreign body. The surrounding marrow proliferates, and the graft is absorbed by giant cells. Callus grows out from the edges of the wound. This may, but usually does not, fill in the defect. In a subsequent paper Barth (1896²) showed that bone ash acts in the same way as bone. He comes to the conclusion that the rapid and efficient formation of new bone depends solely on the presence of calcium salts in the substance which it replaces.

To return to our tumour. We have seen that the first bone is laid down by the fibroblasts of the marrow, and that the connective-tissue fibres are converted into its matrix. We must now inquire into the method by which the bone, once it is laid down, grows at the expense of the dead epithelium. This is partly effected by perforating canals, which allow of invasion and removal of the epithelium by marrow on the other side of the bony trabeculæ, with subsequent ossification. By far the greatest amount of growth takes place in a totally different way. In Plate XXV. Fig. 3 the bone is seen to be directly applied to the calcified tissue. Plate XXV. Fig. 4 is a high-power drawing of such a spot. The bone corpuscles are seen to be in immediate contact with the epithelium. All stages, from free corpuscles to such as are completely enclosed in lacunæ, occur. The latter communicate with the epithelium by means of canaliculi. The bone replaces the calcified tissue without visible absorption. Foreign body giant cells take no part in this change. These appearances are identical with those that occur, according to modern teaching (Schaefer, 1912²²), in normal ossification in cartilage, and with those described by Barth (1895¹) in his grafting experiments with fresh bone. The only process by which this can be brought about is the following. The bone corpuscles, in virtue of being the living cells of the calcified tissue of the body, are able to dissolve the lime salts

and to digest the remains of the dead epithelium. They probably accomplish the former in much the same way as do the giant cells, and the latter by means of ferments. No sooner have they done this than they build up the products of their digestive activity and combine them with the calcium salts they have set free by metabolic processes. The epithelium is used by them solely as a food; none of its organic constituents enter unchanged into the substance of the bony matrix. Epithelium and the matrix of bone are chemically as different tissues as it is possible to find. For this reason, as well as on account of the death of the latter, it appears to me to be inconceivable that any part of it is built up unaltered into the bone substance.

This "ossification in epithelium," if I may use the term, is one of those instances in which pathological processes throw light on those that occur in the healthy body, from which they do not fundamentally differ. It helps to settle two questions on ossification in cartilage about which there is some uncertainty. These are: do the cells of the cartilage become converted into bone corpuscles, and is its calcified matrix directly converted into that of the bone? The answer to the first is: not necessarily, although this change undoubtedly occurs. That to the second is: as bone corpuscles are able to metabolise dead epithelium into osseous matrix, there is no reason to suppose that they cannot do so with cartilage, a tissue which is chemically and developmentally much more closely related to bone.

We have seen that naked bone corpuscles are found among the elements of the dead epithelium (Plate XXV. Fig. 4). Some of these are, no doubt, formed by cell division, and were never enclosed by matrix. Others are the result of solution of the matrix by the cells themselves. There is no evidence for the latter statement in the sections of our tumour, but Pollack (1901¹⁸) found very large bone corpuscles enclosed in wide lacunæ, which he believes to have been produced by absorption. It stands to reason that cells that can build up bone should be able to break it down again.

I have attempted to explain the bone as the necessary result of the stimulus exerted on the fibroblasts of the granulation tissue by the concentrated solution of calcium salts. This tissue serves a useful purpose. It shuts off the calcified dead substance by a ring of calcified living tissue from its surroundings, and thereby neutralises its foreign body action. When its purposes are served, and all the epithelium has been removed, the probabilities are that the bone will disappear in its turn. It is already being eroded by lacunar absorption (Plate XXV. Fig. 3). Maximow (1907¹⁵) states that traces only of bone and of marrow remained in his experiments at the end of a year.

Of the osteomata that have been recorded outside the skeleton there are very few, if any, that are genuine blastomata consisting solely of osseous

tissue. Many are nodules of bone that were formed in connection with calcified areas, the latter of which have been entirely absorbed. Others are teratomata in which the bone preponderates. Yet others are ossified chondromata in association with structures, such as the trachea and the bronchi, that normally contain cartilage. Bony and cartilaginous nodules in the tonsils are to be explained as displaced remnants of the branchial cartilages.

The only question that remains to be answered is, whether the fibroblasts are converted into bone corpuscles by a process of metaplasia, or merely by one of differentiation in an unusual direction? I believe that the latter is the correct answer. In the introduction I have stated my reasons for believing that the cells of granulation tissue represent the nearest approach to an embryonic tissue that is normally found in the body. The fibroblast is an indifferent cell, that has no hard-and-fast structure. Its line of differentiation depends on the part of the body in which it happens to be situated. In the soft parts it becomes a connective-tissue cell, but in the bones it grows into a bone corpuscle. If, now, the conditions in the former tissues come to be identical with those in the latter, the fibroblast follows the line of differentiation which it normally takes in the skeleton. Metaplasia is a term that should be reserved for a metamorphosis, and should not be extended to a simple differentiation. The more highly differentiated a cell is, the more profound is the metamorphosis. In the case of the indifferent fibroblast no such change is possible.

SUMMARY.

1. A type of epithelioma is found in the subcutaneous tissue, which, owing to its poor blood supply, is characterised by slow growth, innocent clinical behaviour, and a marked liability to undergo necrosis and calcification. The probable origin is in a cutaneous germ, which had been displaced at an early period of embryonic life.
2. After its death such a tumour is invaded by granulation tissue, which acquires the structure and functions of marrow, and deposits rings of bone around the calcified tissue.
3. The bone is formed by direct conversion of the fibroblasts of the granulation tissue into bone corpuscles.
4. The first layers of bone are produced as the result of stimulation of the indifferent fibroblasts by the concentrated solution of lime salts. This is dissolved out of the calcified tissue by means of foreign body giant cells. Later the bone grows by invisible absorption of the epithelium. The organic constituents of the latter do not enter directly into the composition of the osseous matrix.
5. This process is analogous to normal ossification in cartilage.
6. It is to be explained as a differentiation, and not a metaplasia of the fibroblasts.

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DESCRIPTION OF PLATES XXIV.-XXV.

PLATE XXIV.

FIG. 1 is a semi-diagrammatic low-power drawing of a longitudinal section through the tumour near its middle. The masses of epithelium are very irregular in shape, and are usually most strongly calcified at their periphery, where they are in contact with living connective tissue. The tumour is composed of two parts. That on the left is the larger. From the capsule a few strands

of coarse fibrous tissue (*c*) extend for a short distance between the epithelial acini. Elsewhere these are separated from each other by empty spaces, produced by liquefaction of the stroma. At *d* there is an old hæmorrhage. In the smaller part of the tumour on the right, the epithelium is much more broken up, and is more irregularly and deeply calcified. At *a* its substance has been invaded by a delicate young fibrous tissue (*b*) from the capsule. Here there are some lobules of fat surrounded by an infiltration with lymphocytes. The young fibrous tissue extends to the point (*b'*). In connection with it some irregular spicules of bone, coloured black in the drawing, have been laid down. At *d* two small, apparently isolated, outgrowths of epithelium are to be seen.

PLATE XXV.

FIG. 2 represents the outer part of one of the columns of the epithelioma, in which the original structure of the tumour is recognisable. Spaces are present between many of the epithelial cells, some of which are traversed by coarse coagulated intercellular processes or prickles. The site of the nucleus in each cell is indicated by an oval or round space. A small cell nest, with a keratinised centre, is shown. Fine granules of calcium salts are present in the majority of the cells. They are most abundant around the nuclear spaces. At the edge of the column there are a few strands of dead fibrous tissue, whose surfaces are encrusted with granules of lime salts. Outside these is an empty space, produced by liquefaction of the stroma.

FIG. 3 is a low-power drawing of a part of the tumour in which bone has made its appearance. At *a.a.*, irregular masses of dead epithelium are shown, at the periphery of which calcification is most intense. *b.b.* are spicules of lamellar bone, with a Haversian canal at *d*. *c* is a small piece of reticular bone. The space surrounding these structures is occupied by a cellular marrow, around the wide blood vessels of which are dense collections of lymphocytes. At *e* a megakaryocyte is seen. Numerous phagocytes, many of which are multinucleated foreign body giant cells, surround the calcified epithelium. In places the extreme edge of the latter, where it comes into contact with the marrow, possesses a thin zone in which the calcium salts have disappeared.

FIG. 4 is drawn from a section stained by Schmorl's method. A piece of lamellar bone is shown, with irregular corpuscles and numerous canaliculi. Some of the fibroblasts of the marrow that are applied to the bone, send processes into its substance. The corpuscles close to the dead epithelium (at the lower right-hand corner of the figure) have established a connection with it by means of canaliculi or by direct application to its surface. Two naked bone corpuscles have invaded the necrotic material.

FIG. 1.

FIG. 3.