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THE REACTION OF BONE TO EXPERIMENTAL CANCER

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In skeletal metastasis of carcinoma it is usual to distinguish between osteoblastic and osteolytic metastases. As a rule, however, new bone is formed and old bone decomposed at the same time, although to a varying extent (*Milch et al.* 1956). Metastases from a prostate cancer, for example, may be both osteolytic and osteoblastic at different sites in one and the same patient (*Jaffe* 1958). In successful endocrine treatment of osteolytic metastases these metastases become osteoblastic (*Coley* 1949). It is not entirely clear how the osteolysis takes place. It has been discussed whether it is the pressure from the tumor which produces necrosis in the bone or whether the cancer cells emit some osteolytic substance. On the other hand, most authors are agreed that few osteoclasts occur in this osteolysis. In experimental tumours *Gorham* (1960, 1964) noted that bone resorption occurs at sites where there is hyperemia on account of their nearness to the tumour, while new bone is formed on the side opposite the tumour, where, according to this author, the blood supply is scanty.

In the present investigation we wished to study in more detail the conditions under which bone resorption and the formation of new bone take place, when a cancer comes into close contact with bone. The experiments were primarily intended to make clear the bone changes caused by the direct invasion of an adjacent cancer (for example, in the pelvis in a case of cancer of the bladder), rather than the changes which arise from bloodborne metastases.

We used the V×2 carcinoma, which can be inoculated homologously into rabbits. It is a squamous-cell cancer and was virus-linked from the beginning. For further information about this cancer, we must refer the reader to *Tjeenberg* (1962). Like *Cerino et al.* (1963), we studied the bone changes, but used a number of different procedures: ordinary X-ray photography, microradiography, conventional histology, investi-

gation of alkaline-phosphatase activity, autradiography using S^{35} and finally investigation by micro-angiography and tetracycline-induced fluorescence.

MATERIAL AND METHODS

Rabbits of mixed breed, weighing between 1 and 3 kg, were used. The contents of a tumour from a rabbit were sucked out or excised. The tumour was crushed to pieces against a fine-meshed metal fabric and then suspended in saline. The suspension was filtered several times through several layers of cotton gauze. It was injected into each rabbit at three places, viz. against the parietal region of the cranium, against the middle part of one femur and intra-osseously through a little hole drilled below the tuberositas tibiae. Approximately 0.2–0.3 ml were injected at each point. In these experiments we did not consider it necessary to administer an exact dose of the tumour material.

Two days before being killed (usually 3 weeks after the start of the experiment), the rabbits received Ledermycin (Lederle) intra-abdominally or intravenously. In most cases micro-angiography with Indian ink or Micropaque was performed in connection with the killing. Polythene tubes were inserted via the aorta abdominalis, partly in the proximal and partly in the caudal direction, and through them the suspension was allowed to drip from a height of 150 cm.

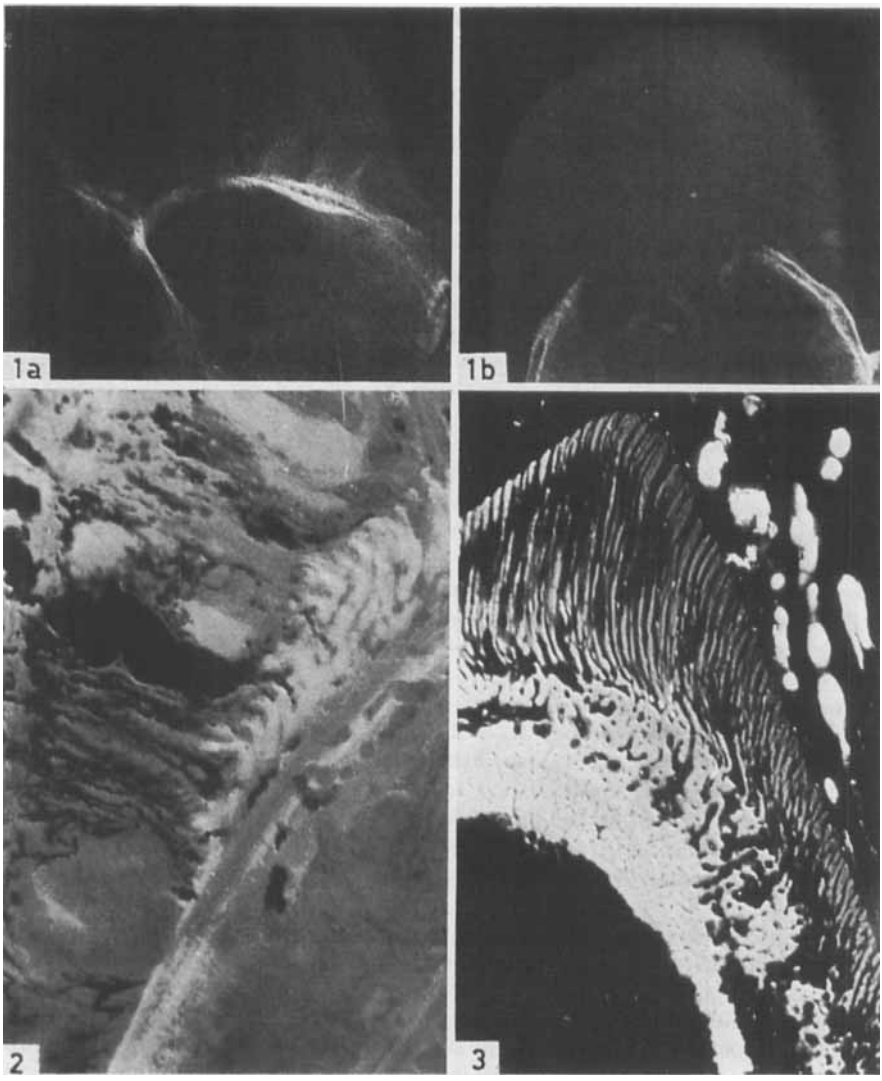
Four rabbits received S^{35} (Radiochemical Centre, Amersham) in a dose of 0.5 millicurie per 100 g body weight. Two of these animals received the dose 5 days before death and the other two 2 hours before death.

After killing, specimens of bone were immediately taken for histochemical and microradiographic examination. Otherwise the animals were deepfrozen at —20° C for 24 hours, after which the tumour bones were sectioned with a saw. X-ray photographs were taken of the entire bones and of the sections. From these photographs specimens were selected for bedding in methyl metacrylate after fixation in alcohol. Slices were sawn off and ground by hand to a thickness of about 125 μ. These were examined by microradiography and then mounted under coverslips for fluorescence microscopy.

Preparations were fixed in neutral formalin for histological examination. Other preparations were fixed in a cold mixture of acetone and alcohol in order to show any alkaline-phosphatase activity. All decalcification was done in a mixture of formic acid and sodium citrate. The preparations were embedded in paraffin wax, sliced into sections 10–15 μ thick and stained with haematoxylin-eosin. For autoradiography with S^{35} the preparations were fixed in formalin and autoradiographed by the stripping-film technique (see *Engfeldt & Westerborn* 1960). A number of sections were pre-stained with haematoxylin, while the rest were unstained. Every eight section in the series was stained in the usual way with haematoxylin-eosin. The Gomori calcium-cobalt method was used to demonstrate alkaline-phosphatase activity.

RESULTS

As early as a week after the inoculation it was possible to palpate tumours at the sites at which the tumour tissue had been injected. The



Figs. 1–3.

Fig. 1 a. Radiograph. Spiculae formation radiating from the cranium, especially in the periphery of the tumour.

Fig. 1 b. Radiograph. Destruction of the cranium in the central part of the tumour.

Fig. 2. Microangiograph. Vessel formation in the outer part of the tumour at right angles to the lamina externa of the parietal bone. The new bone has formed spiculae. Destruction of bone has just started (to the right). Magnification × 8.

Fig. 3. Microradiograph. Transverse section of the tibia. High spiculae formation in the neighbourhood of the tumour. Marked halisteresis and incipient osteolysis. The vessels are filled with Micropaque. Magnification × 8.

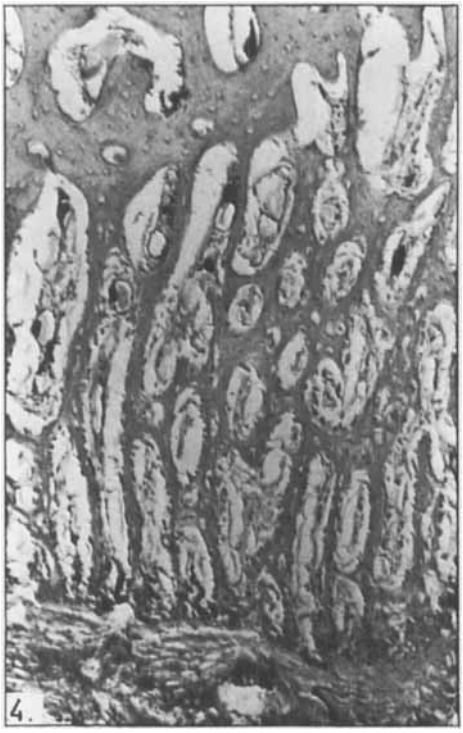


Fig. 4.
Photomicrograph. Haematoxylin-eosin. Periosteal spiculae formation close to tumour growth. Magnification × 50.

tumours grew rapidly and after 3–4 weeks they were so large that in most cases they erupted through the skin. A number of the animals died of marasmus sometime after this. The tumours in the young animals showed a more rapid rate of growth than those in the old animals. As a rule, the animals were killed in the 3rd week and in this connection material was taken under sterile conditions from one of the animals for inoculating into fresh rabbits. In general 3–5 animals were inoculated at a time. From *ordinary X-ray photography* it was easy to see the changes caused by the tumour in the bone. After deep-freezing and sectioning the body, as described above, these changes could be studied in greater detail. In several cases it was possible to see in the cranium distinct spiculae in the outer zone of the tumour. In the central part of the tumour, on the other hand, there was often destruction of the cranium (see Fig. 1 a–b). On the femur the most common changes were large or small cortical deposits on the circumference of the bone. These might be like spiculae in form but were sometimes lamellar. On the tibia both osteoplastic and osteolytic changes were always found. A

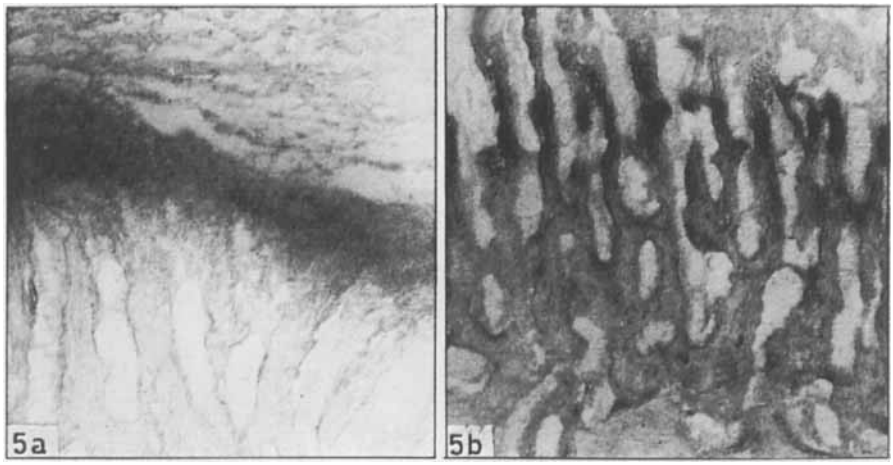


Fig. 5.

a. Autoradiogram. The labelled pre-osteoblast area 2 hours after S^{35} injection. Magnification × 20.

b. Autoradiogram. Five days after S^{35} injection. The bone formed at the time of injection shows up in the band across the spiculae. Magnification × 20.

constant phenomenon was that spiculae were developed to a particularly great extent on the fibula, which was not subjected to any mechanical lesion during the implantation.

The microscopic investigations yielded the following results. The bone proliferation is an initial phase in the bone's reaction to V×2 cancer. It can proceed as long as the surroundings are relatively intact. Bone is formed around capillaries which are directed at right angles to the corticalis or the lamina externa. Fig. 2 shows a microangiogram and a tetracycline fluorescence picture of the cranium with both new formation of bone and incipient destruction of bone. In sections which were taken at right angles to the bone surface this new formation has the appearance of spiculae (Figs. 3 and 4). Spiculae may also develop within the marrow cavity in the direction of the endosteum which probably does not occur otherwise. The osteophytes tend to be larger at the sites of muscle attachments (see Fig. 3 which is a microradiograph).

At the site of bone formation, with short-term labelling with S^{35} (2 hours between injection and death), there is a diffuse labelling of the pre-osteoblast area. This labelling is therefore to be found mainly in the area in front of the growing spiculae (Fig. 5 a). If 5 days are allowed to elapse between the injection of the radio-sulphate and death,

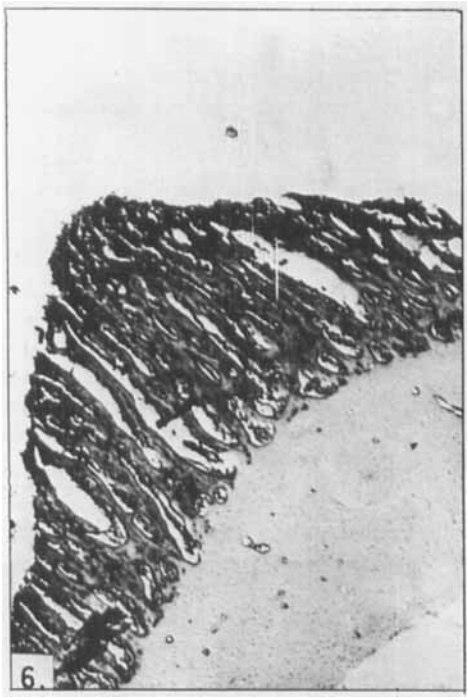


Fig. 6.
Photomicrograph. The alkaline-phosphatase activity well demonstrated in the area of newly formed bone. To the right, the cortical bone with no activity. Magnification × 20.

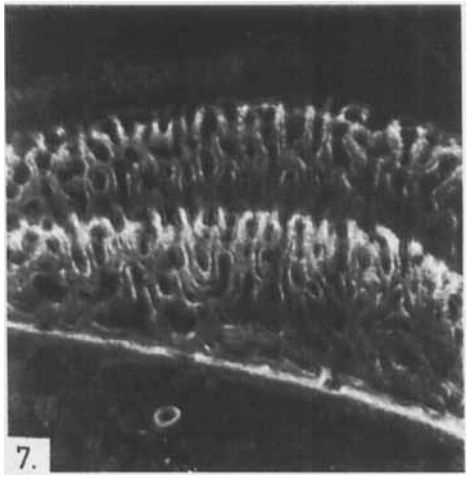


Fig. 7.
Fluorescence photomicrograph from tibia. Fluorescence of two tetracycline injections administered at an interval of 7 days. Magnification × 20.

the sulphur is found instead as a band right across the bone spiculae, at the site corresponding to that of the bone which was in formation at the time of the injection (Fig. 5 b). We find that the area of actual bone formation exhibits great alkaline-phosphatase activity, which is found

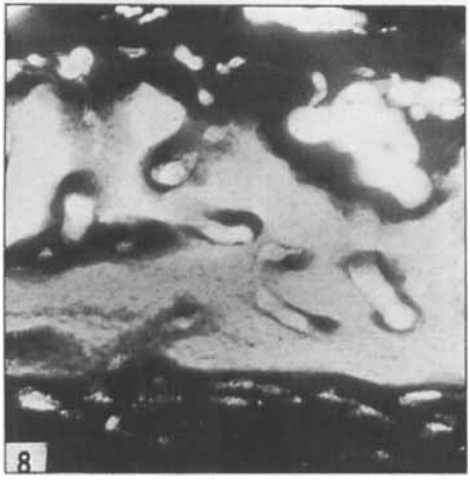


Fig. 8.
Microradiograph with Micropaque-filled vessels from tibia. Dilatation of vascular canals in cortical bone close to the tumour. The bone marrow is seen at the bottom. Magnification × 20.

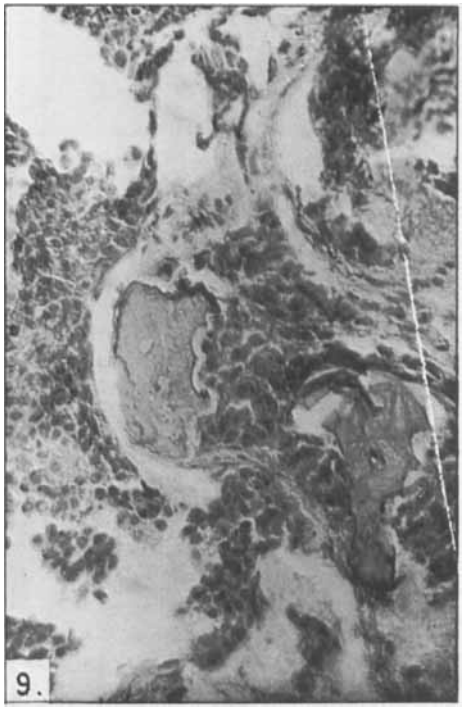


Fig. 9.
Photomicrograph from the cranium. Haematoxylineosin. Residual fragments of bone surrounded by cancer cells. No signs of vessels or osteoclasts.

partly between the bone spiculae and partly in front of these spiculae, in the preosteoblast area. The osteoblasts and osteocytes in newly formed bone also show great alkaline-phosphatase activity (Fig. 6).

The bone which is being newly formed takes up tetracycline, which produces fluorescence in the fluorescence microscope with UV light. On the other hand, no fluorescence is to be found in the original bone. If tetracycline is administered repeatedly, several fluorescence maxima will be found in the growing osteophyte (Fig. 7).

In general it may be said that the new bone is formed in the vicinity of the tumour but not in close contact with it. When the tumour comes too near the newly formed bone and grows through the fibrous wall which separates the spiculae from their surroundings, this bone dies. The tumour cells continue to grow down into the interspaces between the spiculae and often grow within the bone in the vascular canals, often also intravascularly.

Resorption of bone mainly occurs in three ways. In the first place there is a dilatation of the vascular canals in the original bone near the tumour (Fig. 8). This halisteresis is probably caused by the hyperemia. In such cases it is possible to see dilated vessels in the osseous canals. In the relatively well-preserved mesenchyme nearer the tumours, osteoclasts are developed to a certain extent and help to decompose the bone. In the last resort—and this is the most important case—the tumour cells themselves may break down the bone, after it has been cut off from its blood supply. In this connection a diffuse ingrowth of the round tumour cells is found between the residual fragments of bone and by degrees the bone may disappear completely in large areas (Fig. 9).

DISCUSSION

Stimulus to pathological growth or decomposition of bone is not linked with the bone's own tumours, whether they are primary sarcoma or secondary metastases. The present work shows that a tumour—V×2 cancer—which does not have a natural tendency to grow in bone may produce strong reactions in the bone if it is given an opportunity to grow in close contact with the bone. To begin with, there is formation of new bone and then decomposition of bone which may result in the bone disappearing completely. This applies especially when the tumour is inoculated intraosseously in the tibia but also to a great extent if it is applied closely to the cranium. Owing to the fact that the tumour is growing in its vicinity, the bone will probably suffer meta-

bolic damage, which will stimulate the surrounding mesenchyme to take repair measures in the form of the ingrowth of capillaries, which always seem to grow at right angles to the surface of the bone and not towards the tumour. At the same time preosteoblasts are formed in the periosteum or outside the lamina externa of the calvaria in the area of the newly formed capillaries. Then bone is formed round the capillaries, beginning nearest the bone surface, and thereby this bone will have the appearance of spiculae on sections taken at right angles to the bone surface. The bone formed in this way is similar to other new bone, for example, fracture callus. There are, however, certain differences between newly formed bone at the site of the V×2 cancer and the newly formed bone during fracture healing. In a diaphyseal fracture the vessels are mainly directed towards the interfragmental area and even the periosteal vessels show the same tendency. Therefore the periosteal vessels have more acute angles to the corticalis at a distance from the interfragmental area (*Trueta* 1962, *Hulth & Olerud* 1964). The bone spiculae in the vicinity of the cancer may be also formed from the endosteal surface of the diaphysis and from the calvaria. Such a bone growth is never seen in the common type of experimental callus.

The pre-osteoblast area takes up S^{35} in short-term labelling and, if several days are allowed to elapse between the injection and death, the radiosulphate can be found in the fully formed bone as a band right across the bone spiculae. The alkaline-phosphatase activity is also great in areas of bone formation and in the osteoblasts and osteocytes of the newly formed bone. Tetracycline-induced fluorescence is, of course, found in the newly formed bone (but may also be found in resorption cavities).

As the tumour continues its growth, the circulatory connections to the bone are broken off, but the tumour continues to grow into the preformed cavities in the bone. Osteolysis takes place mainly in three ways. As long as the bone is relatively intact, there occurs at a distance from the tumour a hyperemia which results in dilatation of the vascular canals, which contain dilated blood vessels. Nearer the tumour, in areas which have intact connections with the surrounding mesenchyme, there is osteoclastic decomposition. However, this phase is probably relatively short, since the number of osteoclasts is never particularly large. When the bone is entirely disconnected from its surroundings and the tumour has free scope, a very rapid and complete osteolysis takes place, clearly caused by the ingrowing tumour cells themselves. The living tumour cells may conceivably emit substances which dissolve bone.

SUMMARY

When it is inoculated close to the bone or into bone, V×2 cancer produces, to begin with, bone proliferation and then destruction of bone. The bone proliferation is similar to experimental fracture callus and is developed around newly formed capillaries. But these capillaries are mainly directed at right angles to the corticalis, whereas the capillaries in experimental fracture callus are directed towards the haematoma in the interfragmental area. The newly formed bone has a spiculae-like appearance in the X-ray photographs. Such spiculae are also formed on the top of the skull or from the endosteal bone towards the marrow cavity, in which places this kind of new bone formation is otherwise never seen. When the tumour continues its growth, the vascular connections with the bone are completely destroyed and the bone dies and undergoes osteolysis. Before this, however, bone resorption has taken place through halisteresis or osteoclastic decomposition. The greatest destruction seems to be done by the cancer cells themselves.

RESUME

Lorsqu'il est inoculé tout près de l'os ou dans l'os, le cancer V×2 produit pour commencer une prolifération osseuse, puis la destruction de l'os. La prolifération osseuse est similaire au cal des fractures expérimentales et se développe autour de capillaries nouvellement formés. Mais ces capillaries ont principalement une direction à angle droit par rapport à la couche corticale, alors que les capillaires du cal des fractures expérimentales se dirigent vers l'hématome dans la surface interfragmentaire. L'os nouvellement formé présente sur les photographies aux rayons X l'aspect d'un spicule. De tels spicules se forment aussi sur le sommet du crâne ou sur la face intérieure de l'os, du côte de la cavité de la moëlle, endroits où cette nouvelle formation osseuse n'a jamais été observée autrement. Lorsque la tumeur continue à se développer, les relations vasculaires avec l'os sont complètement détruites, l'os meurt et est voué à l'ostéolyse. Toutefois, avant cela, une résorption de l'os s'est effectuée par fonte halistérique ou par décomposition ostéoclaste. La plus forte destruction semble se faire par les cellules cancéreuses elles-mêmes.

ZUSAMMENFASSUNG

Wenn V×2 Cancer nahe am Knochen oder in den Knochen eingepflanzt wird ruft er zuerst Knochenproliferation und dann Knochen-

zerstörung hervor. Die Knochenproliferation gleicht experimentellem Bruchkallus und wird um neugeformte Kapillaren entwickelt. Diese Kapillaren verlauften jedoch hauptsächlich rechtwinkelig zur Corticalis, während die Kapillaren im experimentellen Bruchkallus gegen das Hæmatom im Zwischenbruchgebiete gerichtet sind. Der neugeformte Knochen hat ein spicula-artiges Aussehen im Röntgenbilde. Solche Spicula werden auch am Scheitel das Schädels oder vom endostalen Knochen gegen die Markhöhle hin gebildet, Stellen, an denen diese Art der Knochenneubildung im übrigen niemals gesehen wird. Wenn der Tumor sein Wachtum fortsetzt, werden die Gefässverbindungen mit dem Knochen vollständig zerstört, der Knochen stirbt ab und wird aufgelöst. Ehe dies eintritt hat jedoch Knochenresorption durch Halisterese oder osteoklastische Zersetzung stattgefunden. Die grösste Zerstörung scheint durch Cancerzellen selbst herbeigeführt zu werden.

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