sinus node also may exist. This likelihood is further supported by the fact that tachycardia from glucagon lasted much longer than that from tyramine. The accelerating effects with intranodal glucagon could not be reproduced with comparable amounts of glucagon administered intravenously, indicating that the positive chronotropic action occurred within the sinus node and was not secondary to any effect from recirculated glucagon.

Does glucagon under normal or pathologic conditions have any cardiovascular effect in man? It is known that levels of glucagon in human plasma are increased by hypoglycemia. Many of the symptoms of hypoglycemia are related to elevated circulating catecholamines. Measured levels of glucagon in the fasting state in dogs range from 0-1300 micromicrograms Eq. per ml of plasma(2). During severe hypoglycemia, this concentration may exceed 2000 micromicrograms Eq. per ml or .002 μg Eq. per ml. The positive chronotropic effect in our dogs occurred with concentrations of glucagon which were far greater than those recorded in man. However, secretion of glucagon following hypoglycemia may persist for several hours, thus permitting chronic stimulation of the sinus node. It is possible, therefore, that subliminal levels of glucagon bathing the sinus node over an extended period of time result in a tachycardia, and that some of the cardiovascular symptoms observed during or after hypoglycemic episodes in man may be related to elevated circulating glucagon.

Summary. Glucagon injected into the cannulated sinus node artery of dogs produces a positive chronotropic effect. This effect appears due in part to local release of nodal norepinephrine and in part to a direct action by glucagon on the sinus node.

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Received March 22, 1966. P.S.E.B.M., 1966, v122.

Experience with Intrafemoral Transplant of Breast Tumor in Rats.* (31262)

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A technique whereby tumor cells can be introduced into the medullary cavity of the rat femur has been reported(1). This report describes the successful growth of mammary tumor in the same site.

Method and material. The experiments fell into two phases.

Phase I. Sprague-Dawley female rats were divided into 2 groups of 20. The first group received an homologous suspension of DMBA-induced breast cancer cells. Following the inoculation the tumor failed to grow, was at no time radiologically demonstrable and no viable tumor cells remained when the femora were histologically examined.

The second group received an autologous suspension of DMBA-induced breast cancer cells. All the animals in this group developed lytic lesions in the femur, well shown radiologically and confirmed by histology. The difficulty, however, of establishing a uniform group of animals where inoculation of the femur could be performed in a fixed number,

* This study was supported in part by a research grant from U.S.P.H.S., Nat. Cancer Inst., CA-08219-01 and U.H.F. grant G-65-RP-8.

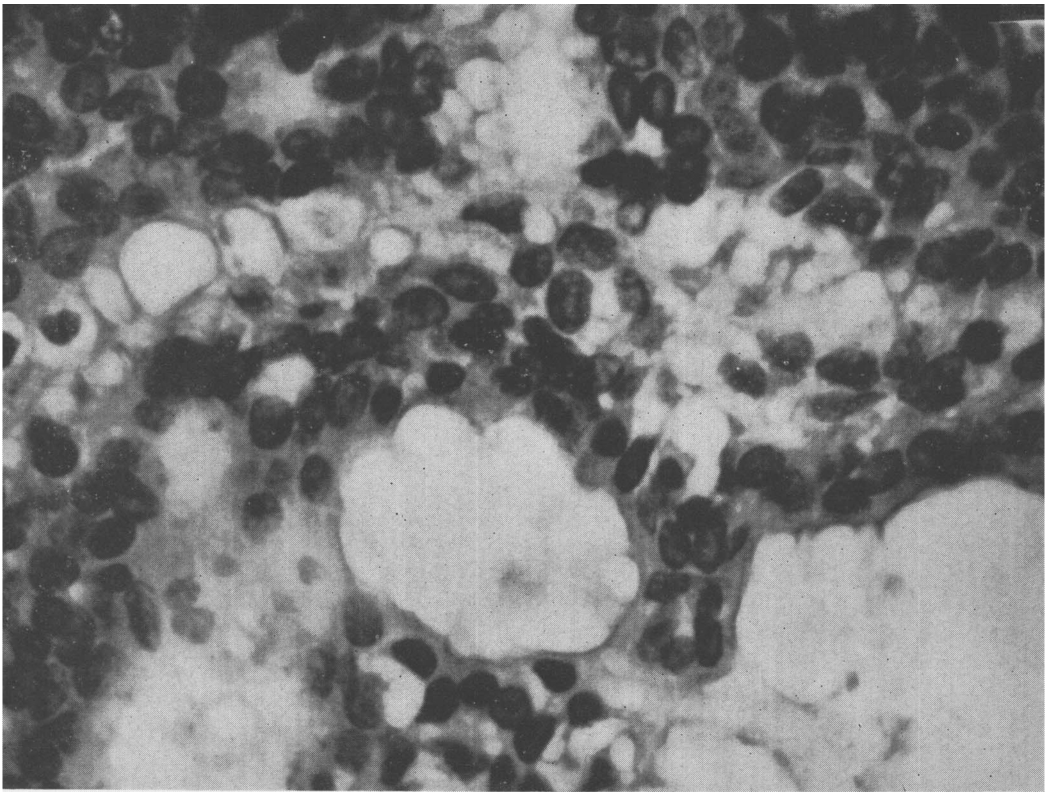

FIG. 1. Histology of the well-established breast adenocarcinoma grown in the Wistar-Furth rat, from which the cell suspension was prepared.

at a fixed time, with the same tumor, led us to seek another strain of animals. The inbred Wistar-Furth rat, in which a well-established breast tumor is easily transplanted and grows within a few weeks, was therefore chosen for the next phase. This allowed preparation of a uniform breast tumor suspension that could be used for inoculation of a group of animals.

Phase II. Two major groups of transplantable breast tumor are available with the Wistar-Furth animals: 1. A highly autonomous tumor. 2. A hormone-dependent tumor. These have been described by Kim(2) and it was decided ideal to see if the transplantable tumors of type 2 could be utilized. Using the Wistar-Furth rats would thus eliminate the induction time of the tumor, a standard cell group could be used for each batch of rats and alterations of hormone environment, drugs and cytotoxic agents on the bone tumor, which is the main aim of the work, could be studied.

The following results are the first in this new group of experiments. To demonstrate the feasibility of growing breast tumor in bone a hormone-sensitive tumor was used. This several-generations transplant was removed from a female Wistar-Furth rat. The histology of this tumor is shown in Fig. 1. The technique of inoculation into the femur was the same as that described in an earlier paper with the following modifications. Penicillin and streptomycin were added to the tissue culture medium to counter infection as a result of faulty technique or obviate the subsequent blood borne infection possible in these animals. The suspension was kept in a small flask at 4°C to depress the metabolic activity of the cells over the period of inoculation and thereby extend their viability. Previously, during the insertion of the cell suspension into the femur of a series of animals, the indicator of the tissue culture medium slowly changed color. The alteration in

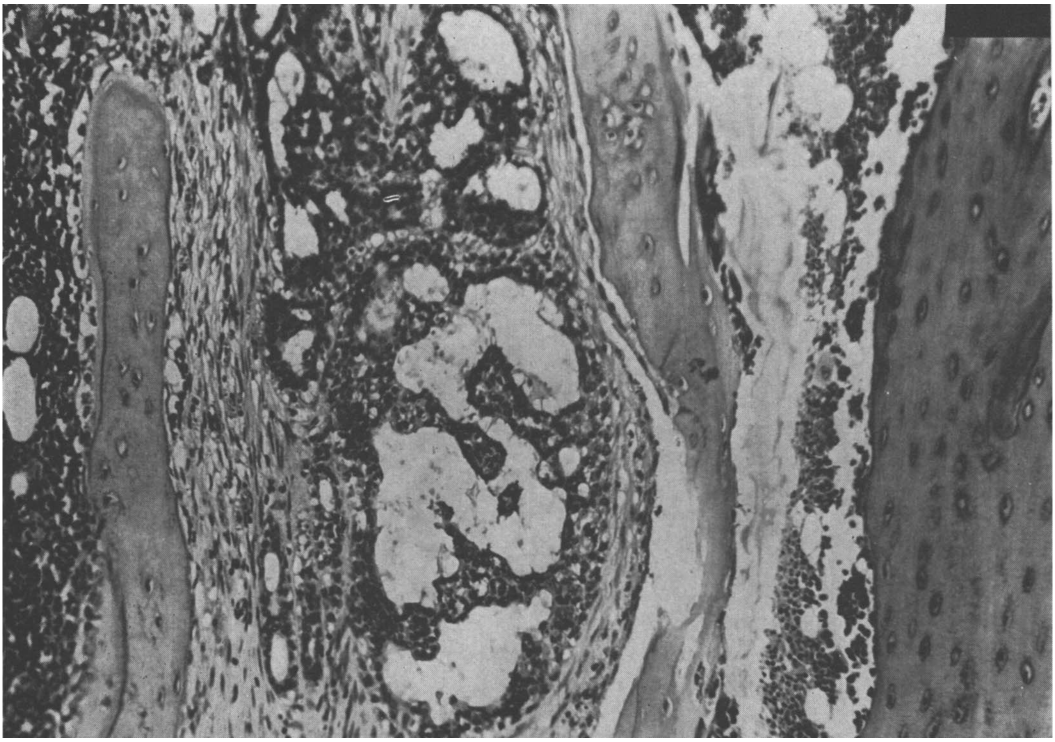

FIG. 2. This portrays the manner in which the breast tumor develops within the bone and forms a tubule of bone around it. Normal bone cortex can be seen framing upper limit of photomicrograph.

the pH of the medium that this suggested has in no way been associated with failure of the transplanted tumor cells to grow. The refinement in procedure, however, was considered advisable because each inoculation is time-consuming and the viability of the cell suspension can be prolonged. When a group of 10 animals receive intrafemoral tumor cells, 15 minutes must be allowed to complete each inoculation, and this must be performed meticulously to avoid contamination.

Experiment 1. A cell suspension was prepared from a transplanted breast tumor taken from an inbred Wistar-Furth rat. This was prepared as on previous occasions by pressing the tumor through the interstices of a wire mesh into tissue culture medium 199 plus penicillin and streptomycin.

This was inoculated into the femoral medulla of a group of 9 animals of which only 6 survived the initial surgery. Unfortunately, the 3 which died were eaten by their cagemates. At the 20th week all 6 of the survivors showed radiological change in the femur which was subsequently confirmed histologically as due to the growth of the transplanted tumor. This "pilot" experiment showed the feasibility of the use of the Wistar-Furth rat and the established transplantable tumor. Furthermore, it appeared to grow in all animals inoculated.

Experiment 2. Ten 60-day-old inbred white Wistar-Furth rats, average weight 194 g, were inoculated with tumor suspension. Of these 10 animals, one died 8 weeks after the inoculation of tumor.

At this time 7 of the surviving 9 animals showed good radiological evidence of tumor growth, were active and gaining weight. Two weeks later these animals underwent a complete bilateral parathyroidectomy to discover if such an ablation might in any way affect the radiological picture of progression of tumor in the bone. There was no evidence of this nor did the growth appear to have been stimulated.

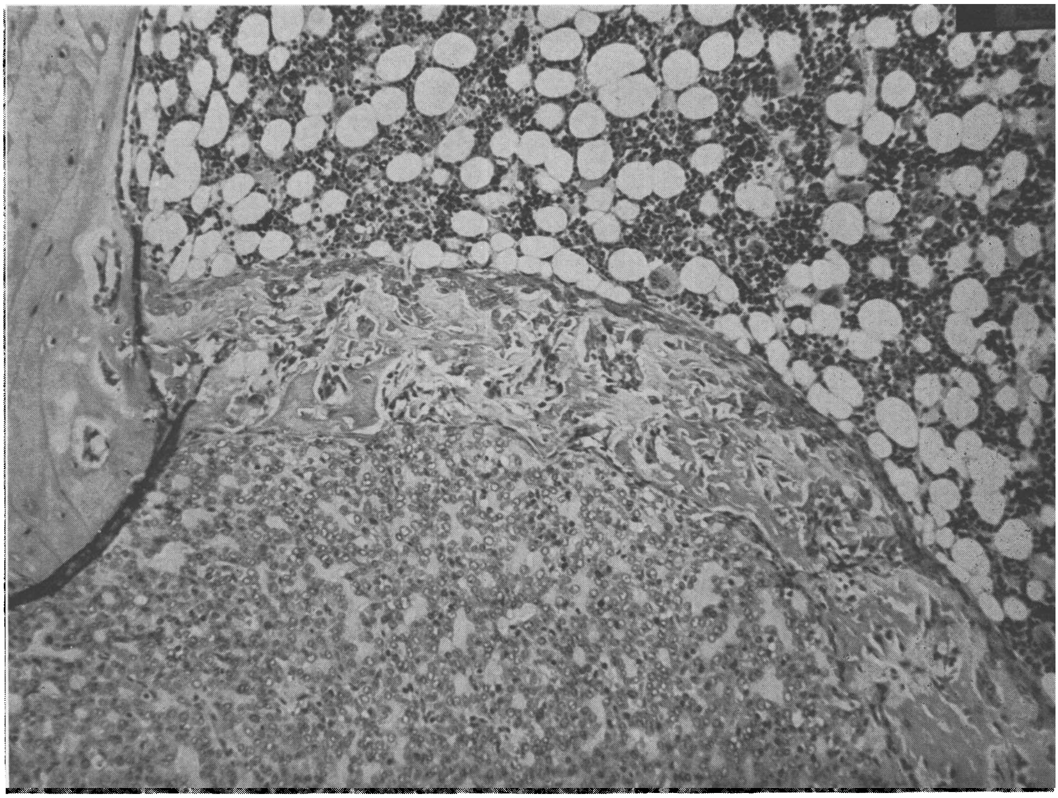

FIG. 3. Showing breast carcinoma to the right with a curved "capsule" of bone preceded by a well-defined zone of osteoblasts. Normal marrow occupies upper photomicrograph and normal bone cortex (which has been broached) the lower left.

Of even greater interest, during the intrafemoral growth of the breast tumor it became apparent that the transplanted cells grew almost invariably in the upper end of the femoral shaft. This was noted in earlier work using auto-transplants of breast tumor in the Sprague-Dawley rat. The tumor is always introduced into the femur *via* a small hole drilled through the intercondylar region after opening the knee joint. After plumbing the marrow cavity with a sound of polythene tubing, active oozing of blood and marrow occurs. After introduction of the cell suspension therefore, the tendency is for the latter to drain away from the upper shaft *via* the hole in the condyle. This is prevented by occlusion with bone wax. One would expect, however, that the subsequent development of the tumor would occur more commonly at the lower end of the bone or in mid-shaft but this is not so.

Experiment 3. A further group of 10 animals was prepared with tumor transplanted to the left leg. All survived the initial surgery and *all* developed tumor visible on X-ray. They were then divided into 2 groups, 5 to undergo oophorectomy and the remaining 5 to receive radiation. Each of the latter received a total dose of 3000 r to the affected left leg.

Of the 5 animals proposed for oophorectomy, one died prior to this operation. The cause of death was pneumonia with an osteomyelitis which I associated with poor technique.

Both groups of animals lost weight following the "treatment," the cause of which was uncertain.

All 4 surviving animals of the oophorectomy group developed well-defined tumor in the bone and 2 of the 4 had lung metastases.

The group of 5 animals in which successful breast tumor growth in the femur was obtained and then irradiated showed exten-

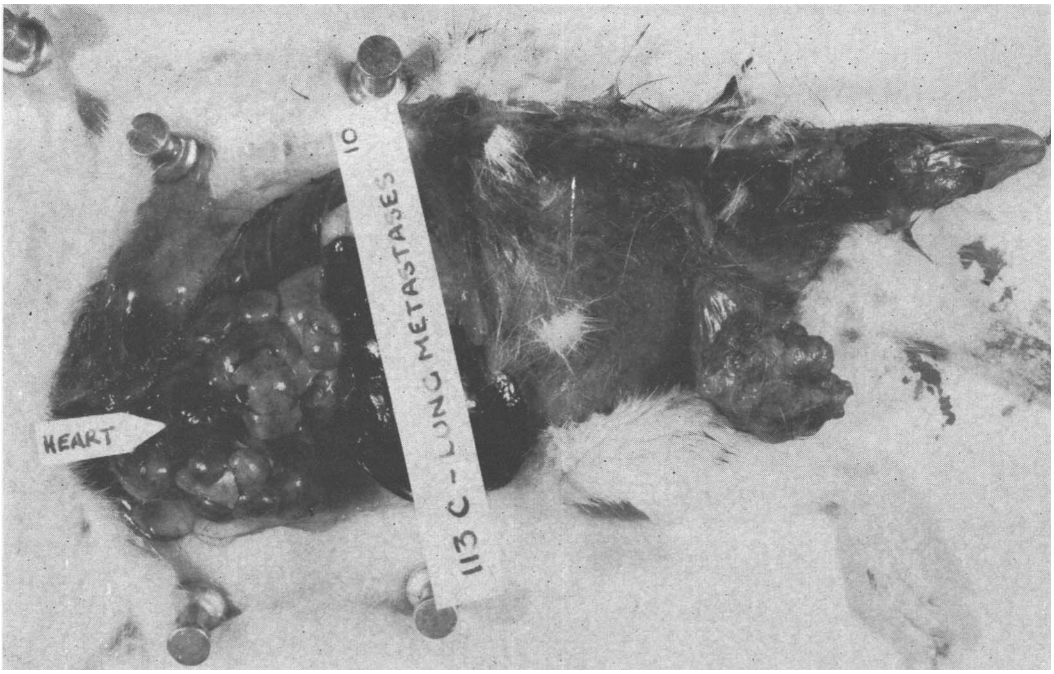

FIG. 4. Lung metastases following inoculation of left femur with a cell suspension of breast carcinoma. See text.

sive fibrosis around the still viable tumor in the bone. The pattern of the tumor was in no way altered but the surrounding fibrosis was immense. Four of these 5 animals had extensive tumor in the lung (Fig. 4).

The tumor in the bone showed a most interesting pattern in each of these 3 experiments. Within the cortex of the shaft, a fringe of normal marrow could be seen on either side of a tubule of new bone which had formed on either side, (or around) the encased breast tumor (Fig. 2). The latter was associated with a small quantity of fibrous tissue. This apparent new-bone formation was not seen in the experiments growing fibrosarcoma in bone nor in the autologous experiments with Sprague-Dawley rats. The formation of new bone has, however, been a constant feature of the transplants in the Wistar-Furth animals. Even when the tumor has exceeded the bounds of the bone cortex that scetion of the tumor expanding within the medulla appears to form a cortex of thin bone about it. It appears as though the advancing solid mass of tumor were condensing bone spicules along its advancing surface. The outer shell of this is rimmed by a well-defined layer of osteoblasts (Fig. 3).

The only difference in the over-all pattern between the irradiated and non-irradiated femora was that in the former, the fibrosis reaction around the tumor acini was markedly increased.

Comment. It is known that the occupation of bone in human breast cancer is associated with both osteolysis and new bone formation. In these experiments using the Wistar-Furth rat new bone formation around growing breast tumor can be clearly demonstrated. It would seem that the tumor is capable of forming an enclosing shell of bone, especially within the marrow cavity. Previous studies introducing non-viable tumor, fibrosarcoma and the performance of the inoculation procedure without tumor suspension have not been associated with any similar osteoblastic activity.

The consistent predilection of the breast tumor for growing in the upper end of the femur is remarkable especially if one considers that this is the area where the lowest density of all is likely using this inoculation procedure.

In 30 animals there was only one where the tumor growth could be demonstrated radiologically as expanding and destroying the bone in the area adjacent to the knee joint, *i.e.*, the lower femur. The breast tumor tended to break out locally from the confines of the bone and did not show the sponge-like expansion seen with fibrosarcoma.

Of all animals undergoing irradiation for the established intrafemoral breast tumor, two features are of interest. First, despite 3000 r to the tumor cells in the leg of a rat, the transplanted tumor was not killed but an immense fibrous reaction occurred about it. The extensive involvement of the lungs in the irradiated animals (Fig. 4) was much in excess of those undergoing oophorectomy although 2 of the latter had small nodular metastases. One would have postulated that if the cells had reached the lungs at the time of the original inoculation then both groups would contain metastases of equal size. It is proposed to pursue this problem further by removal of the left limb immediately after the surgery, and at intervals thereafter in an attempt to show at what phase in the experiment the lungs became involved. It may be that conditioning in an osseous environment enhances metastases to the lungs, or irradiation to the femur may retard the advance of tumor at this site and allow time for the cells that have metastasized to the lungs to appear.

The author would like to thank Dr. Dao for his encouragement and advice in these studies; Dr. Untae Kim for providing the original tumor and helping with interpretation of the histology. Technical assistance from Mrs. B. Bigelow and Sharon Ahrens is gratefully acknowledged.

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Received March 23, 1966. P.S.E.B.M., 1966, v122.

Reversal of Chemotaxis *in vitro* and Chemotactic Activity of Leukocyte Fractions.* (31263)

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Boyden(1) described an *in vitro* technique for measuring quantitatively leukocyte chemotaxis. This method involved inducing granulocyte migration through a filter membrane and counting the cells which reached the lower surface of the filter after a set time interval. Boyden's observation that an active substance placed on both sides of the membrane prevented significant migration suggested that a concentration gradient of chemotactically active substance across the filter membrane was required. The first part of the present study was undertaken to investigate further if chemotaxis, as measured by Boyden's technique, is dependent upon leukocyte response to a continuous concentration gradient of an active substance. Thus, the effect of reversing the concentration gradient of chemotactic serum on leukocytes in the process of migration was determined.

Boyden(1) demonstrated that fresh serum contained a heat labile factor or factors which, when incubated with antigen-antibody complexes, would produce a heat-stable chemotactic agent. Recent studies using this technique have further characterized the conditions under which serum chemotactic substance is produced(2) and have investigated its relationship to serum hemolytic complement(3). These studies suggest that most substances have been demonstrated to have chemotactic activity *in vivo* exert it through a common humoral mechanism. The presence of chemotactic materials in cells or tissues with activity independent of serum is un-

* Supported by Training Grant AI-0128-05 and 06, Nat. Inst. Health, USPHS.