cells was made. Furthermore, 10 animals inoculated only with frozen accelerant and 10 animals inoculated only with fresh (unfrozen) accelerant and allowed to remain unchallenged failed to develop tumors during a 90-day period of observation.

The findings that fresh (unfrozen) cancerous tissues and frozen tumor tissues yielded equally effective accelerative effects upon the growth of the homologous mouse mammary tumor are interpreted as evidence to relate the demonstration by Casey(1-7), and others (13,17), of accelerative factors in frozen tumor tissue and the occurrence of similarly reactive materials in lyophilized tumor tissue, as reported by Snell and his associates (14,15,20,21). Evidently, the accelerative, or "XYZ", factors originate in the tumor cell. It would seem that it is incidental whether the technics for eliciting enhancement employ fresh unfrozen tissues, lyophilized tissues, or frozen cells. However, in contrast to the currently reported unequivocal findings which made it known that fresh and frozen cells were equally productive of accelerative factors, it has been reported for mouse mammary carcinoma 15091a on transfer to C57 mice that the employment of fresh tumor homogenate resulted in an enhancement in only 28 of 62 (45%) mice(16) as compared with evidence for enhancement in 141 of 183 (77%) of the recipients that had been prepared by injections of lyophilized tumor tissues(15). It is suggested that the technic herein described for the preparation of accelerative factors will be equally productive when it is applied to mouse mammary cancer 15091a, irrespective of whether the tissues are employed as fresh, unfrozen cells or after lyophilization.

The failure of various investigators(8-11, 16,18,19) to demonstrate unequivocally the presence of accelerants in *filtrates* of fresh tumor tissue may have resulted, as suggested by Casey *et al.*(9), because "the factors are attached to or parts of a macromolecule which is not filtrable through Berkefeld or Seitz filters." The immunological, chemical, and physical properties, including filtrability, of the fresh (unfrozen) and the frozen accelerants derived from the Z8352 tumor, are under investigation in our laboratories.

Summary and conclusions. (1) In a series of 3 experiments involving 100 ZBC mice an accelerating factor was demonstrated in the unfiltered supernatant fluid which had been prepared by centrifugation repeatedly of a saline extract of fresh (unfrozen) mouse mammary carcinoma Z8352. This tumor, which originated in a female C_3H mouse possessing the milk agent, is maintained by transplantation in ZBC mice not possessing the milk agent. (2) In a second series of 3 experiments involving 102 ZBC mice a similar accelerant was demonstrated in homogenates of Z8352 tumor tissue which had been stored in a deep freeze chest at —18°C for from 63 to 118 days. (3) It is probable that the two factors, the "fresh (unfrozen) accelerant" and the "frozen accelerant", are a single entity derived from the tumor tissue by two different methods.

Received September 12, 1951. P.S.E.B.M., 1951, v78.

Rate of Urinary Calcium Excretion Following Its Intravenous Administration as an Indicator of Bone Metabolism.* (19048)

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The purpose of this study is an attempt to define the state of bone metabolism and some of the mechanisms that maintain calcium and phosphorus homeostasis. The principle of the

* This project has been supported by a cancer control grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

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method is to alter calcium homeostasis acutely by the intravenous administration of calcium and to measure the serum levels of calcium and phosphorus and their renal excretion. The intravenous route eliminates the factor of variability in absorption of calcium from the gastro-intestinal tract. By performing these experiments under identical conditions in all cases, the results obtained in normals and in patients with various types and degrees of bone disease have been compared. While the present study has been performed under metabolic ward control and detailed data were obtained in order to define mechanisms of action, our aim is to evolve a simple and rapid test that will help define the state of bone metabolism at a given period.

Baylor, *et al.*, studied the fate of intravenously administered calcium in three individuals, a normal woman, a patient with postmenopausal osteoporosis, and a man recovering from partial starvation. The calcium retention was greatest in the latter patient, least in the woman with osteoporosis(1).

Method. 50 ml of 10% calcium gluconate solution, containing 446 mg of calcium, are diluted with 500 ml 5% glucose solution in water. The intravenous infusion is given after a 15-hour-fast at the constant rate of 60 drops per minute, so that the total infusion is administered in precisely 4 hours. After the completion of the infusion the patient is given a low calcium diet at regular meal times. Urinary samples are collected by means of an indwelling catheter for one hour before, and at ½, 1, 2, 3, 4, and 5 hours after the start of the infusion. The catheter is then removed and voided urinary samples are collected at 7, 9, 12, 24, and 48 hours after the start of the infusion. By means of a Cournand needle in the femoral artery, blood samples are collected before the infusion and at ¼, ½, 1, 2, 3, and 4 hours after the start of the infusion. The Cournand needle is then removed and subsequent blood samples are obtained by venopuncture at 5, 7, 9, 12, 24, and 48 hours after the start of the infusion. In order to correlate the results of the tests with clinical and metabolic data, the patients are observed on the metabolic ward on a low calcium diet prior to and following the acute experiment. The metabolic and chemical methods employed are described in a previous communication(2).

Results. Twenty adult patients have been studied; one patient was studied twice, another on 4 occasions. The series includes 4 normals and 16 patients with senile osteoporosis, osteomalacia secondary to idiopathic steatorrhea, multiple myeloma, breast carcinoma with osteolytic metastases, prostatic carcinoma with osteoblastic metastases, and cancer patients without clinical or roentgenographic evidence of bone involvement. The procedure caused no significant side reactions; this may be attributed to the slow rate of infusion. Renal function as measured by urea and creatinine clearances, showed no significant change during and after the infusion.

From the data obtained, the cases may be divided into 4 groups based on the urinary calcium excretion per 24 hours after the start of the infusion, (Fig. 1). Group 1, comprised normals, and patients with no demonstrable bone disease; the excretion rate was 302 ± 24 mg/24 hr. In Group 2, the excretion rate

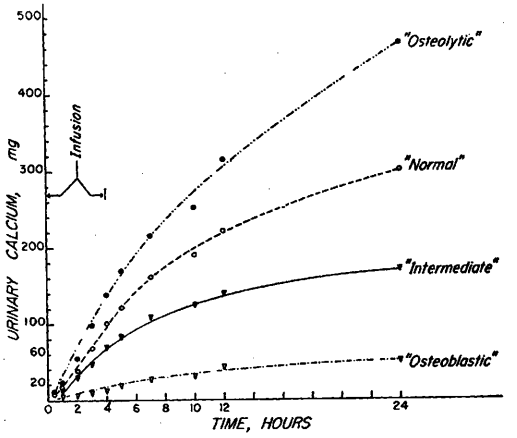

FIG. 1. The urinary calcium excretion during the 4 hr period of intravenous calcium infusion and during 20 hr thereafter. The values represent average cumulative excretion in 20 patients.

1. Baylor, C. H., Van Alstine, H. E., Kentmann, E. H., and Bassett, S. H., *J. Clin. Invest.*, 1950, v29, 1167.

2. Laszlo, D., Schulman, C. A., Bellin, J., Gottesman, E. D. and Schilling, A., *J.A.M.A.*, 1951, in press.

was 50 ± 14 mg/24 hr, indicative of an abnormally great tendency to calcium retention, presumably because of increased bone deposition. In Group 3, comprising patients with osteolytic lesions, the excretion rate was 469 ± 92 mg/24 hr, reflecting their decreased ability to utilize the administered calcium. In Group 4, "intermediate" in character, the rate of calcium excretion was 170 ± 31 mg/24 hr. This group comprised patients who had bone disease in the phase of bone repair, or greater bone formation than one would expect to find in normal adult individuals.

A good correlation has been noted between the calcium and phosphorus metabolic data and the calcium excretion following intravenous infusion, suggesting the usefulness of this method as an adjunct for the metabolic definition of the functional state of the skeletal system. From the data it appears that these excretion rates reflect fluctuations in bone repair earlier and more accurately than do roentgenographic and metabolic studies of the skeletal system. Fig. 2 and 3 illustrate the responsiveness of this method to the fluctuations in bone metabolism induced by therapy or occurring spontaneously. In patient 1, (Fig. 2) the first study was performed at a time when stilbestrol induced a clinical and metabolic remission of an advanced prostatic carcinoma(3). The patient at this time was in metabolic calcium equilibrium on a low calcium diet and the urinary calcium excretion was 17 mg/24 hr. The tendency toward bone deposition was reflected in the subnormal rate of calcium excretion in the acute experiment as illustrated in the lower curve. A second study was performed 5 months after the cessation of therapy, when both clinical and metabolic exacerbation of his disease were evident. At this time the calcium balance was markedly negative and the 24-hour urinary calcium excretion in the metabolic study had risen from 17 mg to 187 mg. Correspondingly, increased rates of calcium excretion were noted in the acute experiment. The reproducibility of the data was studied at this time; on 3 subsequent occasions, when no

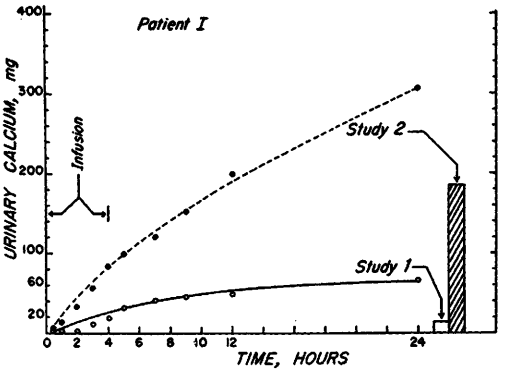

FIG. 2. Urinary calcium excretions on a low calcium diet (bar graph) and following intravenous calcium infusion (curve) in a patient with osteoblastic metastases secondary to prostatic carcinoma. Study 1 was performed during an estrogenic-induced remission. Study 2 was performed during relapse following estrogen withdrawal.

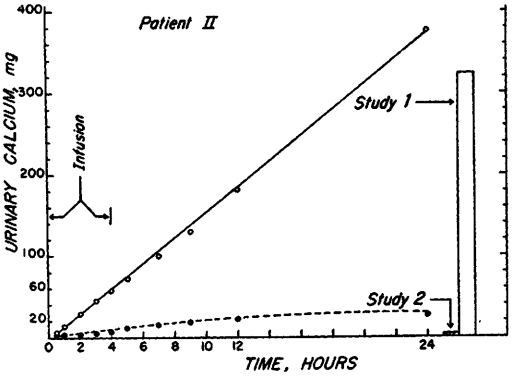

FIG. 3. Urinary calcium excretions on a low calcium diet (bar graph) and following intravenous calcium infusion (curve) in a patient with osteolytic metastases secondary to breast carcinoma. Study 1 was performed during an osteolytic crisis with hypercalcemia. Study 2 was performed during a spontaneous remission.

metabolic fluctuations were observed, similar rates of excretion were found.

In patient 2, (Fig. 3), carcinoma of the breast with osteolytic metastases, the first study shows a phase of abnormally great bone dissolution. This is evidenced by high urinary calcium excretion during metabolic studies, 324 mg/24 hr, as well as by high rates of calcium excretion upon the intravenous administration of calcium. In the second study one month later, a phase of spontaneous bone repair was evidenced by a urinary calcium of 5 mg/24 hr during metabolic studies, a positive calcium balance, and

3. Schilling, A., and Laszlo, D., *J. Clin. Invest.*, 1950, v29, 918

low rates of excretion in the acute experiment.

Renal clearance of calcium and phosphorus are being investigated. Ca^{45} is being used as a tracer for the exogeneously administered calcium. These studies are in progress and will be reported in a subsequent communication.

Summary. The rate of excretion of calcium was studied following its intravenous infusion in normals, and in patients with various bone diseases. Subnormal rates were found in patients with an increased tendency to bone deposition. Increased rates were found in patients with osteolytic activity. The good correlation between these data and metabolic balance studies suggests the usefulness of this test in defining the state of skeletal activity and the effects of therapy thereon.

The authors wish to express their appreciation for the assistance given by Judith Bellin, Estelle D. Gottesman, and Vernice Vankinscott, of the Metabolic Unit.

Received September 12, 1951. P.S.E.B.M., 1951, v78.

Encephalitis in Midwest VI. Western Equine Encepalomyelitis Virus Isolated from *Aedes dorsalis* Meigen. (19049)

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This paper reports the isolation of Western Equine Encephalomyelitis virus (WEE) from each of two batches of *Aedes dorsalis* (Meigen). These viruses were recovered during a study in Weld County, Colo., in 1950 in which specimens of 7 species of mosquitoes were routinely tested for neurotropic viruses. Natural infection of *Aedes* mosquitoes by a neurotropic virus was first reported by Hammon, Reeves, and Galindo(1) with 2 recoveries of western equine encephalomyelitis (WEE) virus from *Aedes dorsalis* (Meigen) in the San Joaquin Valley, Calif., in 1943. The previous year, Hammon and associates (2) failed to recover virus in tests of 4,866 *A. dorsalis* and 2,637 other *Aedes* mosquitoes collected in the Yakima Valley, Washington during an outbreak of encephalitis. On the other hand, WEE virus was isolated 41 times from 9,466 *Curlex tarsalis* Coquillette collected simultaneously with the *Aedes*. Furthermore, Hammon and Reeves(3) state that in tests of 25,162 *Aedes* mosquitoes from 5 western, midwestern, and southwestern states, the western equine virus was isolated only twice in contrast with an infection rate for *C. tarsalis* of 1 of each 225 tested during the 3-year period. It is possible that the vectors and hosts of W.E.E. virus in the midwestern region differ from those in the hot valleys of California. However, only four isolations of virus so far have been reported from mosquitoes in the midwest, three in North Dakota(4,5) and one in Nebraska(6); all were from *C. tarsalis*. The isolations of virus from *A. dorsalis* reported in this paper are the first from the midwest area.

Technics. The mosquitoes were captured by aspirator tube in natural resting places or

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1. Hammon, W. McD., Reeves, W. C., and Galindo, P., *Am. J. Hyg.*, 1945, v42, 299.

2. Hammon, W. McD., Reeves, W. C., Benner, S. R., and Brookman, B., *J. A. M. A.*, 1945, v128, 1133.

3. Hammon, W. McD., and Reeves, W. C., *Am. J. Pub. Health*, 1945, v38, 994.

4. Unpublished record, U.S.P.H.S. Rocky Mountain Laboratory, Hamilton, Montana, vide Jenkins, D. W., *Am. J. Trop. Med.*, 1950, v30, 909.

5. Wenner, H. A., Kamitsuka P., Kramer, M. C., Cockburn, T. A., Price, E. R., *Public Health Reports*, in press.

6. Hammon, W. McD., Reeves, W. C., and Galindo, P., *Am. J. Vet. Res.*, 1946, v6, 145.