reticular cells give a positive reaction for acid phosphatase in acetone fixed sections. There are fewer reactive cells in the cortex than in the medulla. 2. As early as 2 hours after irradiation the mesenchymal reticular cells are mobilized to become active macrophages which stain deeply in the acid phosphatase technic. These become more numerous until at 24 and 36 hours after irradiation the involuted cortex is loaded with macrophages containing ingested lymphocytes or full of vacuoles. At these hours the browned cytoplasm of the epithelial cells is seen as the stroma of the depleted cortex. At 3, 5 and 7 days there is a gradual diminution in number and size of reactive cells. At 9 days the appearance of the thymus is essentially normal.

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Received July 30, 1956. P.S.E.B.M., 1956, v93.

Alterations in Calcium Metabolism in Cancer Patients Treated with 6-Diazo-5-Oxo-L-Norleucine.† (22741)

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6-diazo-5-oxo-L-norleucine (DON), an antibiotic isolated from culture filtrates of a Streptomyces, has been reported by Clarke *et al.* (abstract) to inhibit the growth of sarcoma 180. It is also inhibitive in mouse leukemias and a spectrum of transplantable tumors. In combination with 6-mercaptopurine (6-MP) inhibition of transplantable tumors is synergistic. Patients with cancer metastatic to bone may demonstrate hypercalcemia and hypercalcuria secondary to the osteolysis incident to tumor growth. If the tumor growth is inhibited, as, for example, by local irradiation, the calcium changes may be reversed(1) with the cessation of bone breakdown. Hypercalcemia and hypercalcuria secondary to osteolytic cancer have been corrected also by the administration of adrenal steroids(2) and other compounds. These changes have been regarded as sensitive measures of decreased osteolysis secondary to a reduced rate of growth of tumor in bone(3).

In the course of clinical trials with DON, 11 patients with hypercalcemia and/or hypercalcuria secondary to osteolytic cancer were studied in order to detect any possible tumor inhibitory effects of the compound as measured by the sensitive changes in calcium metabolism. Marked effects on calcium metabolism were seen and it is the purpose of this paper to report these changes.

Material and methods. Six patients had carcinoma of the breast, 2 metastatic carcinoma of unknown origin, 2 bronchogenic carcinoma and one adrenal cortical carcinoma. Hypercalcemia occurred 13 times in these 11 patients. Treatment with DON alone was employed in 8 instances of hypercalcemia and the combination of DON and 6-MP was used in 5 instances. One patient who was normocalcemic but hypercalcuric was treated with DON alone. All but one patient were con-

† This study was supported by Research Grant C1889 from the National Cancer Institute, N.I.H., Public Health Service and by an Institutional Grant from the American Cancer Society.

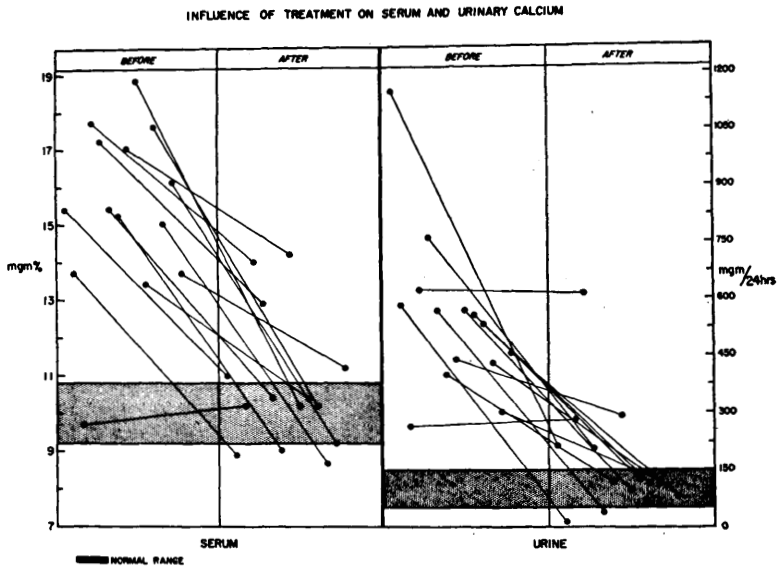

FIG. 1. Influence of treatment on serum and urinary calcium.

fined to bed and all had radiographically demonstrable metastatic cancer in their bones. DON was dissolved in saline in concentrations of 5 to 10 mg/ml. It was administered intravenously or intramuscularly in doses ranging from 0.1 mg/kg/day to 0.6 mg/kg/day. One patient received a dose of 1.2 mg/kg on one day only. Each daily dose was administered as a single injection. The duration of treatment ranged from 9 to 69 days. 6-MP was administered orally in doses of 2.5 mg/kg/day for 12-40 days. Treatment was continued in each patient until signs of toxicity developed (oral mucosal ulcerations, diarrhea, hematological depression).* Intravenous fluids had to be administered to most patients because they were too sick to retain oral feedings. Urinary calciums (method of Fiske and Logan)(4) were determined daily on 24-hour collections. Serum calciums (method of Clarke and Collip)(5) were analyzed approximately 3 times a week. The intake of calcium was restricted to 200 mg/day or less. A complete calcium balance was carried out in 2 patients. Stools and diets were ashed in a muffle furnace at approximately 600°C for 48 hours. Normal calcium ranges: Urine (on 200 mg intake) 50-150 mg/24 hours; serum 9.2-10.8 mg %. Full correction of hypercalcemia and hypercalcuria was regarded as having been achieved if the abnormal values returned to normal ranges regardless of the duration of such changes.

Results. (A) *General observations.* Fig. 1 illustrates the effect of treatment on the levels of serum and urinary calcium. The pre-treatment calcium levels represent the average of several determinations in all instances except when the serum and urinary calcium levels were rising sharply in the period before treatment. In these latter instances the highest pre-treatment value is illustrated. One patient was normocalcemic before treatment but all were hypercalcuric. All post-treatment values are the lowest ones achieved after initiation of treatment. They are not average values.

Serum. Reduction in the serum calcium occurred in each of the 13 instances of hypercalcemia although a return to normal or subnormal calcium levels occurred in only 8 instances (Fig. 1). No change from the normal range occurred in the single normocalcemic patient. The periods of treatment before these changes were seen varied from 7-26 days. The duration of the normocalcemic state was variable lasting from one day to as long as 26 days. In general, recurrences of hypercalcemia were observed when treatment

* Unpublished data.

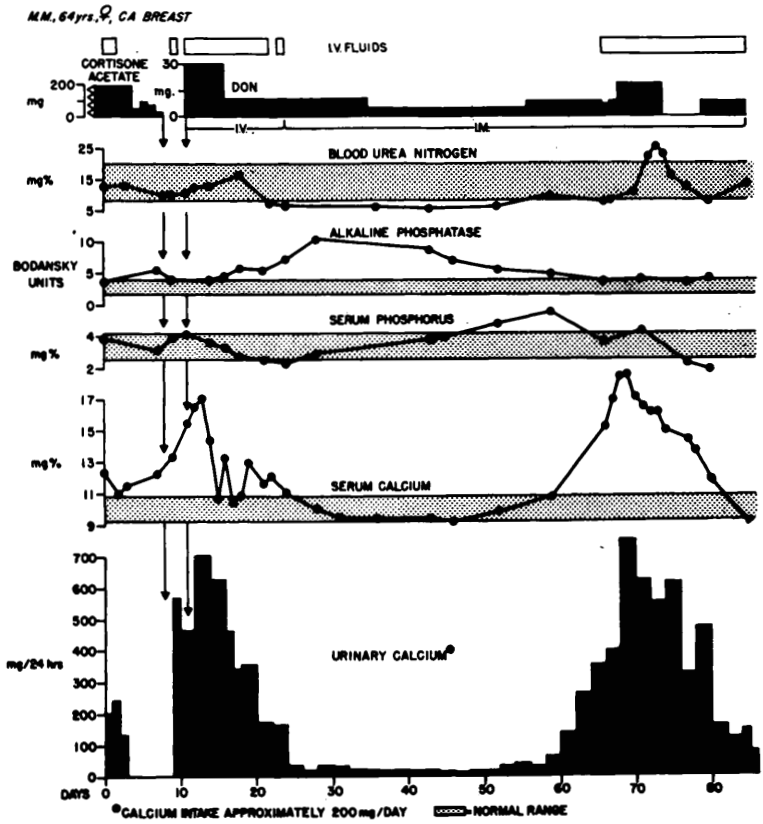

FIG. 2. Sequential changes in serum and urinary calcium in a patient with metastatic breast cancer under treatment with DON.

was stopped but the normocalcemic state persisted in some despite cessation of treatment and hypercalcemia recurred in one patient (Fig. 2) despite continuation of treatment. The decrease in the serum calcium often followed a "double-hump" pattern, that is, an initial decrease to near normal levels was followed by a rise despite continued treatment and then a fall to the normal range (Fig. 2). This pattern probably resulted from the practice of starting with high initial doses of DON (0.6 mg/kg/day) and then tapering to 0.2 mg/kg/day to avoid development of toxicity. In the 3 patients where the serum calcium decreased to lower than normal levels it rose again to the normal range although treatment was continued.

Urine. There was a decrease in the urinary calcium excretion in 12 of the 14 instances of hypercalcuria while 2 patients showed no change (Fig. 1). In 9 of these 12 instances the urinary calcium decreased to normal or lower than normal values. The pattern of response is illustrated in Fig. 2. In general the treatment periods required to produce these changes and the duration of the changes were similar to the serum calcium observations.

(B) *Treatment with DON alone.* There were three instances of full correction of both hypercalcemia and hypercalcuria. Two of these responses occurred in the same patient and are illustrated in Fig. 2. A near-complete response was observed in 2 additional patients whose serum calciums fell to 11.2 mg % and 11.0 mg % respectively with DON treatment. Definite hypercalcemia and hypercalcuria persisted in 3 patients but to a lesser degree than before treatment. There were no consistent differences in the dosages of DON received nor in the toxicity observed between those who responded and those who did not.

In general, the levels of serum and urinary calcium tended to rise promptly in both groups within 1-3 days after treatment with DON was discontinued. One patient, however, was an exception and remained normocalcemic for 22 days after cessation of DON therapy. One patient was normocalcemic but hypercalcuric (250-350 mg/24 hours) throughout the study and DON had no effect in lowering the already normal serum calcium. A total calcium balance was carried out in this patient and no effect on fecal calcium with DON treatment was noted. A total calcium balance was also carried out in another patient and no rise in fecal calcium was observed during DON therapy despite a decrease in the urinary calcium from 1148 mg/24 hours to 214 mg/24 hours over a period of 2 weeks. In one patient (Fig. 2) an attempt was made to prevent recurrence of hypercalcemia by continuing treatment with a maintenance dose of 0.1-0.2 mg/kg/day. Despite this, the hypercalcemia and hypercalcuria recurred but again were fully corrected by increasing the dose of DON.

(C) *Treatment with 6-MP plus DON.* Complete reversals of hypercalcemia and hypercalcuria were observed in 5 instances in the 4 patients in this group. The periods of treatment and total dosages of DON and 6-MP were approximately the same in each patient. In contrast to the DON group, the duration of induced normocalcemia (with one exception) ranged between 15 and 26 days and of normocalcuria between 6 and 32 days. One patient experienced only a 2 day remission in hypercalcemia but a second course of combination treatment resulted in normocalcemia lasting 19 days.

Discussion. The changes in serum and urinary calcium might be explained by a decrease in the rate of bone destruction by tumor owing to the inhibitory effects of the administered DON (and DON plus 6-MP). In view of the experimental evidences of tumor inhibition by these compounds and in view of other studies which suggest that correction of hypercalcemia is evidence of tumor inhibition in bone(1,2,3), this explanation seems most likely. The absence of roentgen evidence of bone healing is somewhat against such an interpretation and in one patient there was an actual increase in the extent of bone disease radiographically during the period between the two episodes of hypercalcemia (Fig. 2). Also disease which was present in soft tissue areas (liver, lung and subcutaneous areas) in some of the patients did not show any objective evidence of regression in conjunction with the calcium changes. All of the patients in this study succumbed to progressive disease and the transient clinical improvement noted in some was largely attributable to correction of hypercalcemia. In general, the addition of 6-MP to the treatment program seemed to exert a longer-lasting effect on the calcium changes noted. 6-MP alone has been used in 4 patients with hypercalcemia and/or hypercalcuria and no substantial effects on calcium metabolism have been observed. These observations are consistent with the demonstrated synergism between DON and 6-MP in transplantable tumors but further clinical studies are necessary in order to establish definite synergism.

Inasmuch as there was a decrease in urinary calcium excretion as the serum calcium fell rather than a calcium diuresis it is unlikely that the observed effects might have been due to enhanced renal clearance of calcium. One balance study revealed no rise in fecal calcium with DON treatment in spite of a marked decrease in urinary calcium, excluding, in this patient at least, the possibility of diversion of calcium excretion from urine to feces, as an explanation for the observed effects.

Chelation of calcium by DON was considered as a remote possibility but *in vitro* studies demonstrated no such action of the compound. Modes of action involving possible inhibition by DON of secretion of hypothetical bone-resorbing substances by the tumor remain entirely speculative at the present time.

Summary. Ten patients with hypercalcemia and hypercalcuria and 1 patient with normocalcemia and hypercalcuria were treated with 6-diazo-5-oxo-L-norleucine alone or in combination with 6-mercaptopurine.

Full correction of hypercalcemia with treatment occurred in 8 of 13 instances in these patients. Correction of hypercalcuria was observed in 9 of 14 instances. Various possible mechanisms underlying these observed effects are considered.

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Received August 1, 1956. P.S.E.B.M., 1956, v93.

Effect of Sodium Lack on Local Response of the Single Crustacean Motor Axon. (22742)

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Sometime ago it was suggested, as the basic assumption for a theory on excitation of nerve (1), that a local or initial depolarization occurred in the immediate area of nerve to which the stimulus was applied. When this local voltage attained a certain optimum value it released or "triggered" the all or none response. Shortly thereafter this hypothetical local potential was measured directly(2) and examined in some detail(3) in crustacean nerve fibers. However, the actual source of this small potential remained obscure. Recently the all or none response has been studied quantitatively by these same investigators and others(4,5), and it has been established that the action potential is the result of an initial rapid sodium ion current inward through the fiber membrane followed by a slower outward potassium ion flow. The question now remains what is the cause of the small local disturbance always observed in the cathodal region with subthreshold stimulation? Is this also due to ionic currents or is it perhaps the result of the sudden appearance of a hormonal substance? It has been suggested, for example, that the release of ACH might produce the initial or local depolarization(6) as it does an end plate potential at the neuromuscular junction.

The present work was undertaken to examine the local response of different single crustacean nerve fibers which possessed quite different excitability properties(7). A brief report of this investigation has already been given in abstract. Recently, certain results were obtained which, because of their significance, are described briefly in this paper.

Methods. The lobster single nerve fiber was used throughout. The preparation, mounting and recording technics have been described (8). The local or initial response was clearly observed with subthreshold stimulation when the fiber was entirely raised in oil, the V method, and stimulation and recording occurred in the identical cathodal area. Cole's physiological solution(9) was used with variations for the study of the effect of different ion concentrations.

Preliminary. One day by accident in mixing the physiological solution the buffer was omitted and although the pH was very low, found later to be only 5.8, the nerve fibers functioned well for several hours. Others, too, have noticed the lack of effect of low pH on these fibers (Adelman, personal communication). The importance of this finding however, was that it meant an entirely sodium-free solution could be applied to a nerve fiber, for some slight amount of sodium has always necessarily been included in the form of buf-

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