RADIOCHEMISTRY AND RADIOPHARMACEUTICALS

Effect of Vitamin D_3, Other Drugs Altering Serum Calcium or Phosphorus Concentrations, and Desoxycorticosterone on the Distribution of Tc-99m Pyrophosphate between Target and Nontarget Tissues

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Radioactive imaging agents are chemically designed for selective distribution. Another approach to selectivity is to find stable compounds that favorably influence this distribution. Using a rat model of myocardial necrosis, we studied effects of various stable compounds (as a single, large dose or fractionated into short series) on the ratio, uptake of Tc-99m pyrophosphate (PPi) by the target lesion/uptake by the principal nontarget, bone (L/B). Vitamin D_3s ability to increase L/B was mediated by the hypercalcemia and hyperphosphatemia that it caused. The hypercalcemia was accompanied by increased [Ca] in the lesion. In contrast, pulse doses of desoxycorticosterone acetate (DOCA) at 7 and 6 hr before killing increased uptake by lesion, increasing L/B from 0.19 ± 0.03 to 0.45 ± 0.08 ($p < 0.01$), with no change in serum [Ca] and minimal changes in serum [P], [Na], and [K]. DOCA also increased the lesion-to-blood ratio from 6.5 ± 0.7 to 15.4 ± 3.9 ($p < 0.05$). These results encourage further study of DOCA's effect and investigation of other stable drugs that may influence distribution of other imaging agents.

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Successful imaging agents must have a high uptake by target tissue and a high ratio of uptake by target to uptake by nontarget tissue. Thus far the main approach to achieving selectivity, i.e., high T/NT, has been to design, so far as is possible, radioactive compounds with highly selective distribution. A second approach, which has received relatively little attention, is to try to influence favorably the distribution of the radioactive compound by concomitant administration of an appropriate stable drug that increases T/NT. Abnormalities in scintigrams are based on functional changes, and most classes of drugs alter function with reasonable selectivity, or they would not be useful as drugs. This approach has the advantage of complementing, rather than competing with the traditional approach to achieving high selectivity of uptake. Depending on circumstances, it may be necessary to administer a given stable drug before, along with, or after the radioactive imaging compound, but in any event the stable drug chosen to modify uptake of the radioactive compound should have its effect within a reasonably short time for maximum clinical usefulness. The drug should also have a high degree of safety. Since the purpose of the drug in this approach is to influence the distribution of a single dose of the radioactive compound, the proposed approach is far more likely to rely upon administration of a pulse (i.e., single large) dose of the stable drug than a long series of doses. This may enhance safety for several classes of drugs.

In a few instances, previous workers have used stable compounds for this purpose. Blau demonstrated the effect of food upon distribution of Se-75-selenomethionine

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in dogs (*1*), and subsequently administered a dose of mixed intestinal hormones (*2*) to modify the distribution of the radioactive compound in man. Other examples are the use of stable perchlorate to block the uptake of pertechnetate by nontarget tissues (*3*), the effect of nitrosoureas on liver and spleen scintigrams (*4*), and of dipyridamole (*5*), bicarbonate (*6*), and grisorixin (*7*) on Tl-201 uptake by heart. These scattered results encourage further study of this general principle.

Tc-99m-labeled pyrophosphate (Tc-99m PPi) and related compounds are useful in the diagnosis of myocardial infarction (*8,9*) but uptake by bone is a significant problem, since the heart lies in a bony cage. In one study of 95 patients with myocardial infarcts, only 9% had greater uptake in lesion than in bone, while in 46% bone uptake was higher than in lesion (*10*). We have shown (*11*) that an i.v. pulse dose of vitamin D_3 (hereafter termed D_3) doubles the ratio of Tc-99m PPi uptake by myocardial lesion to the uptake by bone, a ratio hereafter termed L/B, in rats with 1-day-old lesions; D_3 regularly decreases uptake by bone and often but not invariably increases uptake by lesion. The dose-response curve suggested maximum effect from 1.25 mg (50,000 I.U.) D_3, and the time-action curve was consistent with current views that D_3 requires conversion into metabolites before exerting its effects. The 1.25-mg dose caused acute increases in serum calcium and phosphorus concentrations. Dihydrotachysterol (DHT) also increased L/B.

We now report a study of four questions.

1. Is the D_3 effect, previously shown in rats with 1-day myocardial lesions, also found with older lesions?
2. Are the changes in serum [Ca] and [P] an epiphenomenon or does one or the other (or both) mediate the D_3 effect on uptake?
3. Since myocardial necrosis has been shown to lead to increased [Ca] in the lesion even when no drug affecting serum [Ca] or [P] has been used (*12–14*), would the increase in serum [Ca] or [P] caused by D_3 be likely to exacerbate this abnormality and have adverse effects in the presence of myocardial necrosis?
4. Do other steroids, not in the vitamin D series, also affect L/B?

Concern about possible adverse effects does not stem from the syndrome of vitamin D intoxication that occurred in patients who received very large doses for long periods during the era when such treatment was erroneously considered useful in arthritis, for the minimum time to produce this syndrome was 2 mo of steady daily administration of huge doses (*15*). Any risk from a single pulse dose would be associated with the temporary hypercalcemia or hyperphosphatemia.

MATERIALS AND METHODS

General plan. This has been described previously (*11*). Briefly, myocardial necrosis was created in rats, under ether anesthesia, by the cautery method of Adler (*16*), and the rats were assigned in a random manner to treatment and control groups. Except as otherwise specified below, the subsequent protocol was as follows: At a given time following necrosis, the stable drug was injected in the treatment group; controls received only the vehicle. Two hours before killing, each rat received Tc-99m PPi, 100 μCi/kg i.v.* Blood samples were drawn just before killing with ether. Immediately after death, specimens of bone, heart, kidney, and other tissues were taken. For bone specimens the femora were removed, freed of adherent soft tissue and marrow, and divided into proximal end, shaft, and distal end. The uptake values for both distal femora from a given rat were averaged and are reported here as a single bone value for that rat. Since we have found that proximal and midfemur, as well as tibia and rib, all behave like distal femur in these experiments, data from the last site are reported here as representative of bone. The area of necrosis was dissected from the left ventricle of cauterized hearts; a portion of each sample was placed in formalin, given a code number, and sent for histological examination. The immediately adjacent portion was kept for counting of radioactivity. Specimens of normal myocardium, obtained from cauterized hearts by selecting an area of left ventricle well away from the cauterized site, were similarly treated. All data from lesions presented here were obtained from tissues showing histologically confirmed acute myocardial necrosis.† Radioactivity of all samples was measured in an automatic gamma counter, with appropriate standards. Serum [Ca] and [P] were determined by the Kessler-Gitelman method adapted for SMA-12 (*17,18*); [Na] and [K] were determined by standard flame photometry. Statistical comparisons were by the t-test for unpaired observations.

Effect of D_3 on older lesions. In the first set, myocardial necrosis was created in 26 rats. Twenty-four hours later, 14 rats received, i.v., 1.25 mg of D_3 dissolved in 0.1 ml of absolute ethanol. Twelve controls received 0.1 ml of ethanol i.v. Forty-eight hours after cautery, all rats received Tc-99m PPi and were killed 2 hr thereafter. A second set of 12 treated and 10 control rats was studied in the same way, except that D_3 and Tc-99m PPi were given 72 and 96 hr, respectively, after cautery. Blood and tissues from both sets were studied as described in the general plan.

Effect of changes in serum [Ca] and [P] on L/B. *Increased serum [Ca].* In one set, 13 rats received 0.4 ml of 10% calcium gluconate solution i.p. every 30 min for seven doses, starting 4 hr before killing and continuing until 30 min before killing. We have found that this schedule maintains constant hypercalcemia. Since Tc-99m PPi was given 2 hr before killing, the hypercalcemia was present during the entire uptake period. Twelve controls in this set received 0.4 ml of isotonic

saline solution i.p. on the same schedule. In a second set, nine rats received 0.6 ml of 10% calcium gluconate on the same schedule and eight controls received 0.6 ml of 0.9% NaCl. Blood and tissues from both sets were studied as described under the general plan.

Increased serum [P]. In one set, 14 rats received 1 ml of 200 m*M* phosphate solution (162 m*M* Na_2HPO_4: 38 m*M* KH_2PO_4) i.p. every 30 min for seven doses, beginning 4 hr before killing and continuing until 30 min before killing. We have found that this schedule maintains constant hyperphosphatemia. Since Tc-99m PPi was given 2 hr before killing, the hyperphosphatemia was present during the entire uptake period. Ten controls for this set received 1 ml of isotonic saline solution on the same schedule. In a second set, five rats received 300 m*M* (same Na : K ratio) solution on the same schedule; five controls for this set received the saline solution. Blood and tissues from both sets were studied as described in the general plan.

Decreased Serum [Ca] and [P]. Five sets of four to six rats each received 50 μg/kg of calcitonin in 16% gelatin solution in a volume of 0.5 ml/kg i.m. as a single dose at 4, 2.5, 1.5, 1, or 0.5 hr, respectively, before killing. Five sets of controls, each containing three to five rats, received 0.5 ml/kg of isotonic saline solution i.m. at one of the five times. The remainder of the protocol was as described in the general plan.

Effect of etidronate on L/B. Five sets of four to six rats each received 10 mg/kg of disodium etidronate in 1 ml/kg of isotonic saline solution i.v. as a single dose at 4, 2.5, 1.5, 1, or 0.5 hr, respectively, before killing. Five sets of controls, each containing two to five rats, received 1 ml/kg of isotonic saline at one of the above times. The protocol was otherwise as described in the general plan.

Effect of increased serum [Ca] and [P] on myocardial lesions and on survival. *[Ca] and [P] in lesions.* Two hours after cautery, 44 rats received 1.25 mg of D_3 i.v. Two hours before killing, which occurred 24 hr after administration of D_3, they received Tc-99m PPi. Thirty-five control rats were similarly treated except that the ethanolic vehicle was given instead of D_3. Blood and tissues were studied as described in the general plan but, in addition, samples of normal and necrotic myocardium were prepared for analysis by Subryan's method (*19*). Samples from normal left ventricle and lesion from both control and treated groups were taken for [Ca] and [P] analyses. Samples from three to seven rats were pooled in each crucible. Thus each "n" of 8 in Table 1 refers to eight separate experiments, with three to seven rats used in each. [Ca] was determined by atomic absorption‡ and [P] by the Taussky-Schorr method (*20*).

Survival. Sixteen rats received 1.25 mg of D_3 i.v. 2 hr after cautery, and 16 controls received ethanol vehicle. All were housed in cages containing eight rats each and were observed for 28 days thereafter.

Effect of steroids not in the vitamin D Series on L/B. *Effect of desoxycorticosterone (DOC).* In these experiments the dose (19 mg) was given in four fractions, since we were uncertain of the time course of any possible effect. Thirty-nine rats received 10 mg of DOC in 1 ml of propylene glycol i.m. at 6 hr, plus three doses of 3 mg each of DOC in 0.1 ml of absolute ethanol i.v. at 6, 4, and 2 hr, respectively, before killing. Thirty-nine controls received the vehicles on the same schedule. Since single rats supplied insufficient blood for simultaneous [Ca], [P], [Na], and [K] determinations, blood for [Ca] and [P] was taken from 16 treated rats and 18 controls, while blood for [Na] and [K] was obtained from 14 treated and 15 controls. The protocol was otherwise as described in the general plan.

Effect of desoxycorticosterone acetate (DOCA). In these experiments the pulse does (20 mg) was fractionated into two doses, 1 hr apart. In one set, 13 rats received 10 mg of DOCA in 2 ml of sesame oil i.m. at 19 hr, with a second 10-mg dose at 18 hr before killing. Ten controls for this set received vehicle on the same schedule. In a second set, 13 rats received 10 mg of DOCA at 7 hr and 10 mg at 6 hr before killing, while 13 controls received vehicle. A third set of 14 treated and 12 controls received the same respective doses but the injections were at 3 and 2 hr before killing. Blood samples were taken either for [Ca] and [P], or [Na] and [K], from treated and control animals in each set. The protocol was otherwise as described in the general plan.

RESULTS

All results are expressed here as mean ± standard error of the mean. All values for uptake of radioactivity are expressed as per cent of administered [kg] dose/g of tissue.

Effect of D_3 on older lesions. In the first set of rats, uptake by bone was 0.920 ± 0.106 in controls compared with 0.667 ± 0.069 in treated animals; the uptakes by the (2-day-old) lesion were 0.194 ± 0.028 compared with 0.252 ± 0.078, and the respective L/B values were 0.25 ± 0.04 compared with 0.37 ± 0.06. In the second set, bone uptakes were 0.766 ± 0.097 in controls compared with 0.553 ± 0.089 in treated; the respective uptakes by the (4-day-old) lesion were 0.125 ± 0.019 compared with 0.124 ± 0.021, and the respective L/B were 0.17 ± 0.02 compared with 0.24 ± 0.03 ($p < 0.05$). The effect of D_3 on uptake by the lesion was still present at 2 days but was gone at 4. As expected, the effect on bone was not related to the age of the myocardial lesion, and the change in L/B was still significant at 4 days.

Effect of changes in serum [Ca] and [P] on L/B. *Increased serum [Ca].* In the first set, treated rats, which received 0.4 ml of 10% calcium gluconate, had a serum [Ca] of 12.6 mg/dl, compared with 10.1 mg/dl in controls ($p < 0.025$), a change similar to that produced by

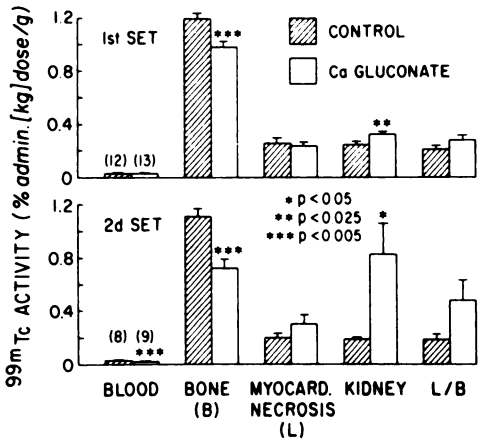

FIG. 1. Effect of hypercalcemia produced by Ca gluconate on Tc-99m PPi uptake in rats. Treated rats received a series of Ca gluconate (10%) injections as described in text; each dose was 0.4 ml (1st set of rats) or 0.6 ml (2nd set). Respective blood values for control and treated rats in second set were 0.034 ± 0.001 and 0.025 ± 0.02 ($p < 0.005$). In this and subsequent figures, respective numbers of rats in treated and control groups are shown in parentheses above blood values, bars represent mean ± s.e.m., and mean for L/B was obtained from mean of individual values of L/B.

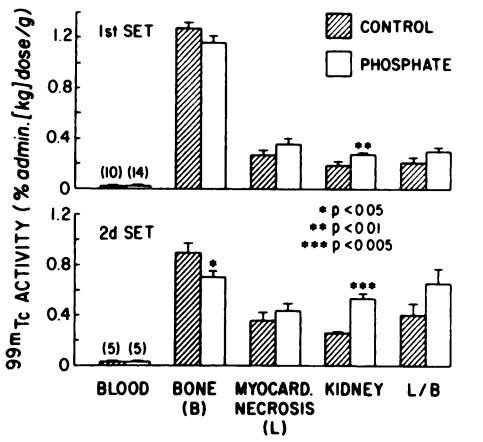

FIG. 2. Effect of hyperphosphatemia (Na_2HPO_4-KH_2PO_4) on Tc-99m PPi uptake in rats. Treated rats received a series of 1 ml phosphate injections as described in text; phosphate solution was 200 m*M* (1st set of rats) or 300 m*M* (2nd set).

a single 1.25 mg dose of D_3 (*11*). Serum [P] was 6.7 mg/dl in treated compared with 7.6 mg/dl in controls, an insignificant change. In the second set, treated rats, which received 0.6 ml of 10% calcium gluconate, showed severe hypercalcemia, reaching as high as 15.0 mg/dl. [P] showed no significant change. The changes in uptake in both sets are shown in Fig. 1.

Increased serum [P]. In the first set, treated rats, which received 200 m*M* phosphate solution, showed a serum [P] of 10.1 mg/dl compared with 8.1 mg/dl in controls, an increase similar to that produced by a single 1.25 mg dose of D_3 (*11*). The serum [Ca] was 8.8 mg/dl in treated compared with 8.6 mg/dl in controls, an insignificant change. In the second set, treated rats, which received 300 m*M* phosphate solution, showed a markedly elevated serum [P] of 15.2 mg/dl, compared with 7.3 mg/dl in controls; serum [Ca] was 6.7 mg/dl in treated compared with 9.1 mg/dl in controls ($p < 0.005$). The changes in uptake in both sets are shown in Fig. 2.

Hypercalcemia per se altered L/B in the direction predicted from our previous results with D_3. Hyperphosphatemia per se altered L/B in the same direction. However, as discussed below, the magnitude of effect from a level of hypercalcemia comparable to that produced by D_3 was less when only serum [Ca] was elevated. The same statement applies to the effect of increased serum [P] when only it was elevated.

Decreased serum [Ca] and [P]. The effect of calcitonin on serum [Ca] and [P] was prompt and sustained. Since all sets of rats showed the same effects, the results have been pooled. The mean [Ca] was 7.8 mg/dl in treated rats compared with 9.8 in controls ($p < 0.005$), while the mean [P] was 5.6 mg/dl compared with 8.2 mg/dl in controls ($p < 0.005$). There was no significant effect on uptake by bone or lesion, or on L/B (Fig. 3).

Effect of etidronate on L/B. Since all sets of rats gave similar results, the results have been pooled (Fig. 4). Etidronate caused no significant effect on uptake by bone or lesion or on L/B. Serum [Ca] was unchanged but serum [P] was 9.3 mg/dl in treated compared with 7.9 mg/dl in controls ($p < 0.005$).

Effect of increased serum [Ca] and [P] on myocardial lesions and on survival. *[Ca] and [P] in lesions.* The results are shown in Table 1. Statistical analysis by Duncan's Multiple Range Test showed that cautery necrosis increased [Ca], as previously described, in ischemic myocardium (*12-14*). D_3 caused further increase in [Ca] in lesion but not in normal myocardium. Neither necrosis nor D_3 changed tissue [P].

Survival. Fifteen of the 16 treated rats and 15 of the 16 controls survived for 28 days. None of the surviving rats in either group appeared in poor condition at the end of this time.

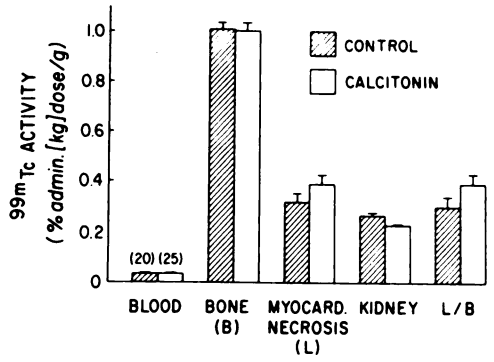

FIG. 3. Effect of hypocalcemia and hypophosphatemia produced by calcitonin (see text) on Tc-99m PPi uptake in rats. Differences are not significant.

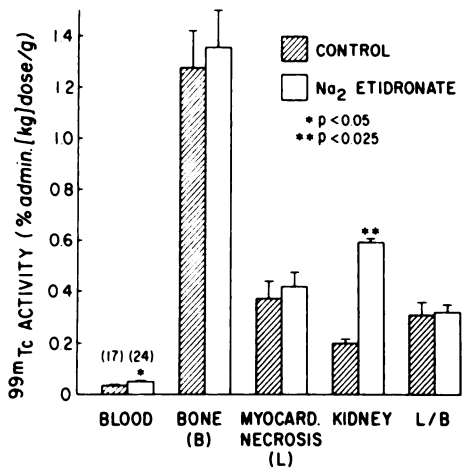

FIG. 4. Effect of Na_2 etidronate (see text) on Tc-99m PPi uptake in rats. Respective blood values for control and treated rats were 0.036 ± 0.003 and 0.051 ± 0.005 ($p < 0.05$).

Effect of steroids not in the vitamin D series on L/B. *Effect on desoxycorticosterone (DOC).* The results are shown in Table 2. DOC had no effect on serum [Ca] or [P]. The pulse dose of DOC also had no effect on serum [Na], while [K] showed a slight but significant increase. Especially noteworthy is the increase in L/B caused by DOC in the absence of any increase in serum [Ca] or [P].

Effect of desoxycorticosterone acetate (DOCA). The results are shown in Fig. 5. There was no change in serum [Ca] in any of the sets. Treated values compared with respective controls were: first set (last dose of DOCA at 18 hr before death) [Ca] 9.2 ± 0.2 mg/dl compared with 9.2 ± 0.3 mg/dl; second set (last dose of DOCA at 6 hr before death) [Ca] 9.2 ± 0.2 compared with 9.2 ± 0.2; third set (last dose of DOCA at 2 hr before death) [Ca] 8.9 ± 0.04 compared with 8.9 ± 0.1. For serum [P] the treated values compared with respective control were: first set 7.4 ± 0.2 mg/dl compared with 7.2 ± 0.3 mg/dl; second set 8.7 ± 0.8 compared with 9.4 ± 0.2; and third set 8.2 ± 0.1 compared with 7.7 ± 0.1. The changes in the first and second sets were not significant, but the change in the third set was ($p < 0.05$). Serum [Na] increased slightly but significantly in the first set from 143 ± 1 mEq/l in controls to 145 ± 0.5 mEq/l in treated ($p < 0.05$), and in the third set from 145 ± 1 to 149 ± 1 ($p < 0.01$). It did not change significantly in the second set. Serum [K] increased slightly but significantly in the second set from 3.5 ± 0 mEq/l to 3.7 ± 0.1 mEq/l ($p < 0.05$) and did not change in the other sets. Despite the minimal nature of all these changes—and, in particular, the absence of any change in serum [Ca]—L/B increased significantly in the second and third sets of rats. This was primarily due to increased uptake by lesion.

DISCUSSION

In this study a single pulse dose, or two closely spaced doses, of an appropriate stable drug favorably influenced the distribution of a subsequently administered imaging compound. This has previously been accomplished (*5–7*) for imaging agents that reveal infarcts as decreased foci of radioactivity, but encouraging examples of the use of this approach with agents that show increased uptakes in lesions are few. If one assumes, *a priori*, that it should be easier to influence the function of normal than of abnormal tissue,‖ the two situations pose somewhat different problems. With agents used in scintigrams that show minimal uptake by the lesions of interest and concentrate in the normal tissue of the organ of interest, the nontarget tissues that are of concern because of possible interference are other adjacent normal tissues, e.g., overlying or surrounding organs that also have significant uptake. To exert favorable influence, the stable drug must selectively influence uptake among normal tissues. Agents used in scintigrams for focally increased activity are taken up by the lesions of interest, and the only significant role of normal tissues is as interfering nontargets. Here, to be successful, the stable

TABLE 1. EFFECT OF 1.25 mg VITAMIN D_3 I.v. ON [Ca] AND [P] IN NORMAL AND NECROTIC MYOCARDIUM

Treatment	n*	[Ca]‡ in normal myocardium (μg/g)	[Ca]‡ in necrotic myocardium (μg/g)	[P] in normal myocardium (μg/g)	[P] in necrotic myocardium (μg/g)
Control	8	70 ± 17	265 ± 19†	877 ± 114	658 ± 48†
D_3	8	64 ± 14	516 ± 104	826 ± 97	725 ± 94

* See text.

† In one of the eight crucibles analyzed, five of the six rats contributing necrotic tissue to the pool had histologically confirmed necrosis, and the histologic specimen from the remaining rat was lost. Otherwise all lesions in all crucibles represented histologically confirmed necrosis.

‡ Differences in myocardial [Ca] between: control normal and control necrosis $p < 0.05$; D_3 normal and D_3 necrosis $p < 0.05$; control normal and D_3 normal n.s.; control necrosis and D_3 necrosis $p < 0.05$. All [P] differences n.s.

TABLE 2. EFFECT OF DOC* ON UPTAKE OF Tc-99m PYROPHOSPHATE AND ON SERUM [Ca], [P], [Na], AND [K] IN RATS

Treatment	n	Uptake (% kg dose/g) Bone (B)	Myocardial necrosis (L)	L/B
Control	39	1.097 ± 0.037	0.299 ± 0.029	0.27 ± 0.02
DOC*	39	0.944 ± 0.049†	0.346 ± 0.024	0.41 ± 0.04‡

	n	[Ca] mg/dl	[P] mg/dl	n	[Na] (mEq/l)	[K] (mEq/l)
Control	18	9.5 ± 0.1	7.7 ± 0.1	15	145 ± 1	3.9 ± 0.1
DOC*	16	9.1 ± 0.2	7.1 ± 0.3	14	145 ± 1	4.5 ± 0.1‡

* Desoxycorticosterone.
† $p < 0.05$.
‡ $p < 0.005$.

drug must influence the ratio of uptake by lesion to uptake by normal nontarget tissue, either by decreasing the denominator (*3*) or increasing the numerator. Although the ideal effect would be to do both, increase in the numerator is especially desirable since absolute uptake is important per se. Although increasing the uptake by lesions, according to the aforementioned assumption, may be the more difficult task, it has now been shown possible, at least in the model used here.

We chose cautery to create a model lesion of myocardial necrosis in rats for several reasons. This technique reliably produces a well-demarcated lesion, permitting comparison between clearly normal and clearly necrotic myocardium; the uptake of Tc-99m PPi and related compounds is increased in this model lesion as in clinical infarcts; other investigators have found this model useful in testing uptake of various compounds as possible imaging agents for myocardial infarcts (*21*). We have also found in the present study that the increased tissue [Ca] found in ischemic infarcts is found in this model.

In the model used here to search for possible improvements in a diagnostic technique, the drugs showing promise thus far have been D_3 and dihydrotachysterol, as well as DOC and DOCA—all steroids. Although other classes of drugs may subsequently be found to affect this model, some analogies with past developments in the pharmacology of two other classes of steroids, used to improve therapy rather than diagnosis, are of interest. Glucocorticoids, in doses several hundred times that needed in adrenal insufficiency, are useful in treating certain other disorders and have provided benefits that were not easily predictable from previous knowledge of adrenal physiology; this has also contributed to our knowledge of pathological mechanisms, e.g., the role of autoimmunity in certain diseases. Moreover, single-pulse doses of glucocorticoid 400 times the dose needed in adrenal insufficiency have been used in certain human disorders without adverse effect (*22*), although continued use of large doses often causes serious toxic effects. Similarly, large doses of estrogens in males, achieving levels in vivo far beyond those normally found even in females, are useful in treatment of certain neoplasms, a result that was not automatically predictable from normal estrogen physiology and has given new insight into hormonal control of neoplasms. Adverse effects often follow such chronic administration, but single very large doses of estrogens (e.g., accidental ingestion of large numbers of oral contraceptive tablets by children) have been reported to cause few adverse effects. Returning to our present model, used in an attempt to improve a diagnostic rather than therapeutic technique, our results were not easily predictable from the known effects

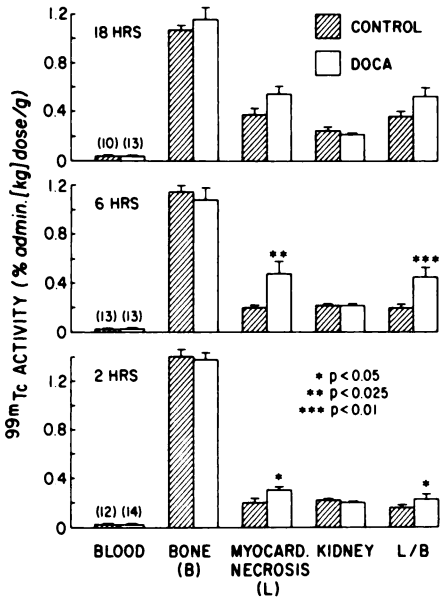

FIG. 5. Effect of desoxycorticosterone acetate (see text) given at various times on Tc-99m PPi uptake in rats. Times shown at upper left of each section of figure are times between last dose of DOCA and killing.

of "physiologic" doses of the steroids we used. We shall consider possible mechanisms of effect, benefit, and toxicity of D_3 and of DOCA as used here.

Our results suggest that the increase in L/B caused by D_3 is mediated, at least in part, by increased serum [Ca]. However, the pulse dose of D_3 that we used increased [Ca] only moderately, to values around 12 mg/dl (*11*); this degree of hypercalcemia alone, when produced by calcium gluconate and thus unaccompanied by hyperphosphatemia, changed L/B in the same direction as D_3, but not to the same extent. In the pulse dose we used, D_3 increased serum [P] to approximately 11 mg/dl in rats (*11*); this represents only a modest elevation in rats, since serum [P] in this species is normally higher than in man. Hyperphosphatemia of this degree, when produced by injection of phosphate solution and thus unaccompanied by hypercalcemia, also increased L/B in the same direction but not to the same extent as D_3. To achieve, by raising only serum [Ca] or only serum [P], the same magnitude of effect on L/B as that produced by D_3 required much higher respective elevations of [Ca] and [P] than those produced by D_3. These findings are consistent with the view that the *combination* of increased serum [Ca] and serum [P] that D_3 causes is important in its effect on L/B.

The analytical method for serum [P] used here is not specific for phosphate ions. The "artificial" increase in serum [P] produced by administration of a very large dose of the phosphorus-containing compound, etidronate, did not exert any effect on uptakes, in contrast to "genuine" hyperphosphatemia.

Phosphate complexes appear to accumulate below the osteoid layer at the calcified front of bone. The manner in which increased serum [Ca] and [P] decreases this uptake needs further study. The increased renal radioactivity we often observed may suggest that a diuretic effect of increased serum [Ca] or [P] caused decreased blood radioactivity and decreased bone uptake, but this hypothesis has many flaws. Renal radioactivity at any one time does not necessarily indicate renal excretion over a period of time; blood radioactivity was, in fact, more often higher than lower after D_3 in our experiments; marked drop in bone uptake has sometimes occurred in our studies without increased renal radioactivity; and etidronate raised renal radioactivity without lowering bone uptake.

Although D_3 decreased uptake of Tc-99m PPi by bone more regularly than it increased uptake of Tc-99m PPi by lesion, the latter effect was sufficiently frequent to merit attention. The hypothesis that phosphate complexes accumulate in infarcts and other sites of recent necrosis because of increased calcium in these sites is attractive but debatable. Studies in McArdle's syndrome (*23*) and rhabdomyolysis (*24*) suggest that calcium accumulation (*12–14*) is important. Siegel (*25*) showed that dihydrotachysterol increased uptake of Tc-99m hydroxyethylidene diphosphonate in ischemic myopathy in rats, increasing [Ca] in both blood and lesion. But other work (*26,27*) does not support the view that increased [Ca] in the lesion is necessary for increased uptake. Moreover, intramuscular injection of iron into rats (*28*) and man (*29*) increases the uptake of Tc-99m phosphate complexes at the injection sites. Our results are consistent with the view that increased uptake by lesion, when caused by D_3, is mediated at least in part by increased serum [Ca], which causes still further accumulation of Ca in the lesion. The role of increased [P] is more difficult to explain, since we did not find increased [P] in the lesion, despite the fact that serum from the rats used for analysis of [Ca] and [P] in the lesion after receiving D_3 was also analyzed for Ca and P and showed the expected increases in both [Ca] and [P].

Thus, the ability of D_3 to double L/B offers encouragement to the general approach used here. Another possible benefit from the use of an appropriate stable drug with scintigraphic agents may be the ability to provide, through comparison of results with and without administration of the stable drug, additional information about the age or nature of the lesion. In a few instances, e.g., the use of dexamethasone in association with adrenal scintigraphy (*30*), this principle has already been successfully exploited. Since the problem of distinguishing between a recent myocardial infarct and a persisting image of an older infarct sometimes arises clinically, the association between the effect of D_3 on uptake by our model lesion and the age of the lesion is of interest.

But an important aspect of the ability of D_3 to increased [Ca] in serum and lesions is the question of possible adverse effect. Increased [Ca] in lesions may simply reflect injury without itself causing damage, but previous workers (*31–33*) have suggested that calcium influx may itself contribute to cell death. However, it has not been clearly established that brief hypercalcemia is deleterious in patients with myocardial necrosis. Indeed, administration of calcium is recommended in certain arrhythmias, e.g., those caused by hyperkalemia (*34*). Also, it has long been known (*35*) that administration of calcium salts is efficacious in some instances of cardiac arrest, and this use is still recommended (*36*). Thus far, pulse doses of D_3 (and of DOCA) have been well tolerated by conscious rats without apparent adverse effects, and the pulse dose of D_3 did not increase mortality in the small series we studied. The question thus remains unresolved and deserves further study before consideration of clinical use of D_3 to improve diagnosis of myocardial infarction. Probably the least controversial point concerns patients receiving cardiac glycosides. Evidence has accumulated over many years (*37–41*) that hypercalcemia increases, and hypocalcemia decreases, the cardiac toxicity of these drugs.

Since D_3 decreased bone uptake of Tc-99m PPi, the

question arises whether increased serum [Ca] or [P] from other causes, as sometimes seen clinically, may significantly affect bone scintigrams. This is possible, but the problem is mitigated by the fact that most clinical conditions that raise serum [Ca] do not simultaneously raise serum [P], and vice versa. We did not find any effect of decreased serum [Ca] and [P] on uptake.

In contrast to D_3, DOCA did not affect the serum [Ca], despite DOCA's clearly favorable effect on L/B. Our initial reason for studying adrenal steroids was to explore the effect of glucocorticoids, which are known to decrease bone-matrix formation, at least in long-term use, and to produce negative calcium balance. Cortisol had no effect on L/B, and corticosterone—the rat's principal adrenal steroid—had only questionable effect. Extending this study to DOC, we obtained the somewhat surprising results shown above, which led to further study with the more convenient DOCA. Whereas D_3 increased L/B by regularly decreasing uptake by bone and sometimes increasing uptake by lesion, DOCA increased L/B by regularly increasing uptake by lesion. The magnitude of DOCA's effect on L/B was similar to that of D_3. In rats killed 6 hr after DOCA the ratio, radioactivity of lesion/radioactivity of blood (L/blood), was significantly increased from 6.5 ± 0.7 to 15.4 ± 3.9 ($p < 0.05$). There was also a trend ($0.10 > p > 0.05$) toward an increase at 2 and 18 hr: The respective changes were from 7.8 ± 1.0 to 10.6 ± 1.8, and from 11.1 ± 1.2 to 15.3 ± 1.6. This favorable effect on a second target/nontarget ratio is encouraging in view of the importance of blood as a nontarget in myocardial scintigrams. Neither D_3 nor hypercalcemia alone, nor hyperphosphatemia alone, significantly changed L/blood. A third ratio of interest, radioactivity of lesion over radioactivity of normal myocardium, showed a trend in that it appeared increased in all three sets of rats receiving DOCA, but the increase was not significant in any. No other treatment changed this ratio.

The mechanism of DOCA's effect on uptake of Tc-99m PPi by lesion is not known and its explanation must await further work. DOCA is known to act at extrarenal sites as well as at the distal nephron, but those extrarenal sites described thus far (*42*) do not explain our results. The pulse dose of DOCA caused slight but significant elevation of serum [Na] in some experiments, but the magnitude of this acute change was not enough to suggest that it would cause serious adverse effect. No decrease in serum [K] was observed. The slight but, in one experiment, significant increase in serum [K] is puzzling, but may represent temporary efflux of K from red cells into serum.

Production of focal myocardial necrosis in rats by repeated very large daily doses of mineralocorticoids has been long known (*43*) and extensively studied (*12,44-46*), but the effect requires, in addition to the prolonged steroid administration, supplementary measures such as a potassium-deficient diet or drastic catharsis. There is good evidence (*43,45*) that the necrosis is brought about by severe potassium deficiency. Such prolonged drastic treatment is reported to make the animals severely ill, with marked weight loss and often renal as well as cardiac lesions (*12*). It is difficult to relate these previous experiments to the effect we observed from brief, well-tolerated administration of DOCA.

Before any approach developed in rats can be considered for clinical trial, other species must obviously be studied. Our results to date suggest that DOCA may be a more promising compound than D_3 because DOCA does not produce hypercalcemia, and thus the unresolved question of possible clinical problems resulting from the temporary hypercalcemia caused by D_3 (see above) does not arise with DOCA. Moreover, the time required for D_3 to affect uptakes after its injection is longer (*11*) than for DOCA, another advantage of the latter from the standpoint of possible eventual clinical usefulness.

FOOTNOTES

* Kindly supplied by Dr. Jehuda Steinbach.

† Except in the 28-day study, any rat in which histologic examination failed to confirm myocardial necrosis was discarded from the study. Thus the numbers of treated and control rats in each set refer to animals with confirmed necrosis. See also footnote to Table 1.

‡ We wish to thank the Erie County Clinical Chemistry Laboratories for the atomic absorption analyses.

‖ The great difficulty in increasing uptake of radioactive iodide by thyrotropin stimulation of poorly differential thyroid neoplasms, compared with the results obtained with normal thyroid tissue or well-differentiated thyroid neoplasms, is one example supporting this assumption.

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REFERENCES

1. BLAU M, MANSKE RF: The pancreas specificity of Se^{75}-selenomethionine. *J Nucl Med* 2:102-105, 1961
2. BLAU M, MANSKE RF, BENDER MA: Clinical experience with Se^{75}-selenomethionine for pancreas visualization. *J Nucl Med* 3:202, 1962 (abst)
3. LATHROP KA, HARPER PV: Biologic behavior of ^{99m}Tc from ^{99m}Tc-pertechnetate ion. *Prog Nucl Med* 1:145-162, 1972
4. KAPLAN WD, DRUM DE, LOKICH JJ: The effect of cancer chemotherapeutic agents on the liver-spleen scan. *J Nucl Med* 21:84-87, 1980
5. HAMILTON GW, NARAHARA KA, YEE H, et al: Myocardial imaging with thallium-201: effect of cardiac drugs on myocardial images and absolute tissue distribution. *J Nucl Med* 19:10-16, 1978
6. HETZEL KR, WESTERMAN BR, QUINN JL III, et al: Myocardial uptake of thallium-201 augmented with bicarbonate: concise communication. *J Nucl Med* 18:24-25, 1977
7. MAUBLANT J, GACHON P, MOINS N, et al: Myocardial imaging in dogs with thallium-201 and the ionophore grisorixin. *J Nucl Med* 21:787-789, 1980

8. BONTE FJ, PARKEY RW, GRAHAM KD, et al: A new method for radionuclide imaging of myocardial infarcts. *Radiology* 110:473-474, 1974
9. PARKEY RW, BONTE FJ, MEYER SL, et al: A new method for radionuclide imaging of acute myocardial infarction in humans. *Circulation* 50:540-546, 1974
10. CUARON A, ACERO AP, CARDENAS M, et al: Interobserver variability in the interpretation of myocardial images with Tc-99m-labeled diphosphonate and pyrophosphate. *J Nucl Med* 21:1-9, 1980
11. CARR EA JR, CARROLL M, MONTES M: The use of adjunctive drugs to alter uptake of ^{99m}Tc-Sn-pyrophosphate by myocardial lesions and bone. *Life Sci* 22:1261-1274, 1978
12. D'AGOSTINO AN: An electron microscopic study of cardiac necrosis produced by 9 α-fluorocortisol and sodium phosphate. *Am J Pathol* 45:633-644, 1964
13. D'AGOSTINO AN, CHIGA M: Mitochondrial mineralization in human myocardium. *Am J Clin Pathol* 53:820-824, 1970
14. SHEN AC, JENNINGS RB: Kinetics of calcium accumulation in acute myocardial ischemic injury. *Am J Pathol* 67:441-452, 1972
15. HOLMAN CB: Roentgenologic manifestations of vitamin D intoxication. *Radiology* 59:805-816, 1952
16. ADLER N, CAMIN LL, SHULKIN P: Rat model for acute myocardial infarction: application to technetium-labeled glucoheptonate, tetracycline and polyphosphate. *J Nucl Med* 17:203-207, 1976
17. KESSLER G, WOLFMAN M: An automated procedure for the simultaneous determination of calcium and phosphorus. *Clin Chem* 10:686-703, 1964
18. GITELMAN HJ: An improved automated procedure for the determination of calcium in biological specimens. *Anal Biochem* 18:520-531, 1967
19. SUBRYAN VL, SOLOMONS CC, GORDON SG, et al: A micromethod for the quantitative analysis of calcium, magnesium, and phosphorus in human skin. *J Lab Clin Med* 86: 1056-1060, 1975
20. TAUSSKY HH, SHORR E: A microcolorimetric method for the determination of inorganic phosphorus. *J Biol Chem* 202:675-685, 1953
21. DAVIS MA, HOLMAN BL, CARMEL AN: Evaluation of radiopharmaceuticals sequestered by acutely damaged myocardium. *J Nucl Med* 17:911-917, 1976
22. TURCOTTE JG, FEDUSKA NJ, CARPENTER EW, et al: Rejection crises in human renal transplant recipients. Control with high dose methylprednisolone therapy. *Arch Surg* 105:230-236, 1972
23. SWIFT TR, BROWN M: Tc-99m pyrophosphate muscle labeling in McArdle syndrome. *J Nucl Med* 19:295-297, 1978
24. SILBERSTEIN EB, BOVE KE: Visualization of alcohol-induced rhabdomyolysis; a correlative radiotracer, histochemical, and electron-microscopic study. *J Nucl Med* 20:127-129, 1979
25. SIEGEL BA, ENGEL WK, DERRER EC: Localization of technetium-99m diphosphonate in acutely injured muscle. Relationship to muscle calcium deposition. *Neurology* 27: 230-238, 1977
26. DEWANJEE MK, KAHN PC: Mechanism of localization of ^{99m}Tc-labeled pyrophosphate and tetracycline in infarcted myocardium. *J Nucl Med* 17:639-646, 1976
27. KRONENBERG MW, ETTINGER UR, WILSON GA, et al: A comparison of radiotracer and biochemical methods for the quantitation of experimental myocardial infarct weight: in vitro relationships. *J Nucl Med* 20:224-231, 1979
28. JONES AG, FRANCIS MD, DAVIS MA: Bone scanning: radionuclidic reaction mechanisms. *Semin Nucl Med* 6:3-18, 1976
29. BYUN HH, RODMAN SG, CHUNG KE: Soft-tissue concentration of ^{99m}Tc-phosphates associated with injections of iron dextran complex. *J Nucl Med* 17:374-375, 1976
30. GROSS MD, FREITAS JE, SWANSON DP, et al: The normal dexamethasone-suppression adrenal scintiscan. *J Nucl Med* 20:1131-1135, 1979
31. WROGEMANN K, PENA SDJ: Mitochondrial calcium overload: a general mechanism for cell-necrosis in muscle diseases. *Lancet* 1:672-674, 1976
32. TRUMP BF, MERGNER WJ, KAHNG MW, et al: Studies on the subcellular pathophysiology of ischemia. *Circulation* 53 (Suppl. 1):I-17-I-26, 1976
33. SCHANNE FAX, KANE AB, YOUNG EE, et al: Calcium dependence of toxic cell death: a final common pathway. *Science* 206:700-702, 1979
34. ETTINGER PO, REGAN TJ, OLDEWURTEL HA: Hyperkalemia, cardiac conduction and the electrocardiogram: a review. *Am Heart J* 88:360-371, 1974
35. KAY JH, BLALOCK A: The use of calcium chloride in the treatment of cardiac arrest in patients. *Surg Gynecol Obstet* 93:97-102, 1951
36. MELMON KL, MORRELLI HF, Eds: *Clinical Pharmacology: Basic Principles in Therapeutics*. 2d ed, New York, Macmillan Publishing Co., 1978, p 257
37. BOWER JO, MENGLE HAK: The additive effect of calcium and digitalis. A warning, with a report of two deaths. *JAMA* 106:1151-1153, 1936
38. GUBNER RS, KALLMAN H: Treatment of digitalis toxicity by chelation of serum calcium. *Am J Med Sci* 234:136-143, 1957
39. COHEN BD, SPRITZ N, LUBASH GD, et al: Use of a calcium chelating agent (NaEDTA) in cardiac arrhythmias. *Circulation* 19:918-927, 1959
40. ELIOT RS, BLOUNT SG: Calcium, chelates, and digitalis. A clinical study. *Am Heart J* 62:7-21, 1961
41. MODELL W: Clinical pharmacology of digitalis materials. *Clin Pharmacol Ther* 2:177-190, 1961
42. MULROW PJ, FORMAN BH: The tissue effects of mineralocorticoids. *Am J Med* 53:561-572, 1972
43. DARROW DC, MILLER HC: The production of cardiac lesions by repeated injections of desoxycorticosterone acetate. *J Clin Invest* 21:601-611, 1942
44. SELYE H: *The Chemical Prevention of Cardiac Necrosis*. New York, Ronald Press, 1958, pp 54-113
45. NICKERSON M, KARR GW, DRESEL PE: Pathogenesis of "electrolyte-steroid-cardiopathy". *Circ Res* 9:209-217, 1961
46. LEHR D, KRUKOWSKI M: About the mechanism of myocardial necrosis induced by sodium phosphate and adrenal corticoid overdosage. *Ann NY Acad Sci* 105:137-182, 1963