PREVENTION OF EXPERIMENTAL HYPERPARATHYROIDISM BY MAGNESIUM AND POTASSIUM SALTS[1]

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ABSTRACT

Young, growing rats were treated with parathyroid extract and NaH_2PO_4. This treatment produced an unusually intense osteoclastic bone absorption. However, in animals which, in addition, received either $MgCl_2$ or KCl, the bone destruction was diminished and, often, osteoblastic new-bone formation occurred. Still, the skeleton never became totally insensitive to the action of the parathyroid extract.

On the other hand, the production by parathyroid hormone plus NaH_2PO_4 of pathologic calcium deposits in the heart and kidney was completely inhibited by the magnesium and potassium salts, at certain dose levels.

It might have been thought that the protective effects of magnesium chloride and potassium chloride are merely due to some local interference with the absorption of the phosphate, which accentuates the action of parathyroid hormone. However, it could be shown that, even when NaH_2PO_4 is given subcutaneously (by the granuloma pouch technique), both $MgCl_2$ and KCl afford protection against parathyroid hormone overdosage.

The protective effect of potassium and magnesium salts appears to be closely linked to the presence of an excess of phosphate. In rats not sensitized with NaH_2PO_4, parathyroid hormone actually increased susceptibility to the specific toxic effects of $MgCl_2$ and KCl, so that many animals died, exhibiting neuromuscular disturbances and brain edema.

It is assumed that NaH_2PO_4 sensitizes, while $MgCl_2$ and KCl desensitize, the body to certain toxic actions of parathyroid hormone, particularly to its ability to produce soft-tissue calcification. On the other hand, the hormone actually decreases the tolerance of the organism to some of the toxic actions (especially the neuromuscular effects) of $MgCl_2$- and KCl-overdosage, unless these particular effects are counteracted by concurrent treatment with NaH_2PO_4.

The experiments furnish additional examples of the selective conditioning by electrolytes for certain hormone effects.

IT WAS recently noted that combined treatment with certain corticoids and NaH_2PO_4 regularly produces large myocardial necroses in rats (1, 2) and that the development of these lesions can be prevented by the concurrent administration of $MgCl_2$ or KCl (3). Combined treatment with

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dihydrotachysterol and NaH_2PO_4 produces an acute, purulent myocarditis, accompanied by the Mönckeberg-type of arteriosclerosis; these changes can likewise be prevented by the simultaneous administration of $MgCl_2$ or KCl (4). Since dihydrotachysterol shares many of the pharmacologic actions of parathyroid hormone, the question arose whether treatment with magnesium or potassium salts could also suppress the manifestations of intoxication produced by this hormone, especially in animals sensitized to it by concurrent treatment with NaH_2PO_4.

MATERIALS AND METHODS

Two hundred-twenty female Wistar rats, with a mean initial body-weight of 50 gm. (range: 38–60 gm.), were subdivided into the 22 equal groups which constitute the three experimental series of this study.

The parathyroid extract used was a special preparation of "Paroidin" (Parke, Davis and Company), and contained 550 I.U./ml.

The salts employed were monosodium phosphate "Reagent" ($NaH_2PO_4 \cdot H_2O$, Fisher Scientific Company), potassium chloride "Reagent" (KCl, Fisher Scientific Company), and magnesium chloride "Reagent" ($MgCl_2 \cdot 6H_2O$, Merck and Co., Inc.). The two last named salts were invariably given by stomach tube, but the route of administration of the NaH_2PO_4 varied, as indicated in the text.

Throughout the experiment all animals were kept exclusively on "Purina fox chow" and tap water.

The first experiment was terminated after four days, the other two after seven days of treatment, by killing all the survivors with chloroform. Immediately after autopsy the heart and kidneys were fixed in neutral formalin, for subsequent staining with hematoxylin-phloxine (to determine the general histologic structure) and with von Kossa's silver nitrate technique (for the histochemical demonstration of calcium). In addition, the distal metaphyses of the femurs, as well as samples of the ribs of each animal, were fixed and simultaneously decalcified in Susa solution, for subsequent staining with hematoxylin-phloxine. All tissues were embedded in paraffin. The intensity of the calcium deposition in the hearts and kidneys, as well as the severity of the osteitis fibrosa (in the femurs and costochondral junctions) were graded in terms of arbitrary scales of 0 to 3. The means of these determinations (with standard errors), as well as the mortality rate, are listed in the Tables.

EXPERIMENTAL

First Experiment

In this series, parathyroid hormone was administered at the daily dose of 0.5 ml., subcutaneously, twice daily, while the NaH_2PO_4, $MgCl_2$ and KCl were all given at the dose of 150 mg., in 2 ml. of water, twice daily, by stomach tube, as indicated in Table 1.

At this high dose level, parathyroid hormone in itself sufficed to produce osteitis fibrosa and marked calcium deposition in the hearts and kidneys, but it did not cause any mortality (group I). Concurrent treatment with NaH_2PO_4 aggravated the osteitis fibrosa only insignificantly, while the cardiac and renal calcification produced by the parathyroid hormone were significantly augmented so that an 80% mortality resulted (Group II). On the other hand, although the rats which received $MgCl_2$ (Group III),

TABLE 1. EFFECT OF ORAL TREATMENT WITH NaH_2PO_4, $MgCl_2$ AND KCl UPON PARATHYROID HORMONE (Ptr-H) ADMINISTRATION (FIRST EXPERIMENT)

Group	Treatment	Severity of lesions (Scale: 0–3)			Mortality (%)
		Bones	Heart	Kidney	
I	Ptr-H	2.0±0.3	1.1±0.25	1.1±0.2	0
II	Ptr-H+NaH_2PO_4	2.4±0.3	1.9±0.28	2.8±0.1	80
III	Ptr-H+NaH_2PO_4+$MgCl_2$	2.1±0.4	0	0	10
IV	Ptr-H+NaH_2PO_4+KCl	2.0±0.25	0	0.3±0.2	50

or KCl (Group IV) in addition to parathyroid hormone and NaH_2PO_4 still exhibited marked osteitis fibrosa, they developed little or no calcium deposition in hearts and kidneys, and their mortality rate was definitely reduced.

The cardiac changes in Groups I and II consisted mainly of calcium deposition in the blood-vessel walls and muscle fibers (Fig. 1). Often, silver-nitrate-tingible calcium granules were clearly visible in apparently still well preserved muscle cells, but in some instances, patches of myocardial nec-

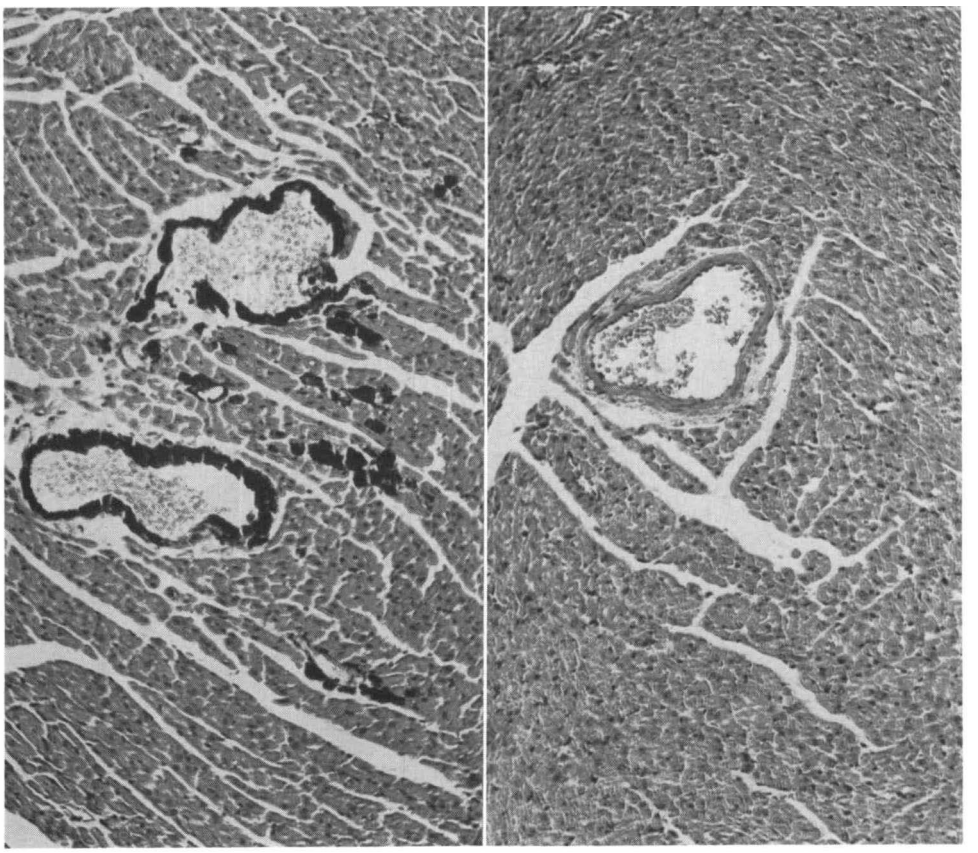

FIG. 1. Intense calcium deposition in the walls of two vessels, as well as in some of the myocardial fibers of a rat treated with parathyroid hormone plus NaH_2PO_4 (left), in comparison with the essentially normal heart of a rat which, in addition to parathyroid hormone plus NaH_2PO_4, received $MgCl_2$ (von Kossa's stain ×120).

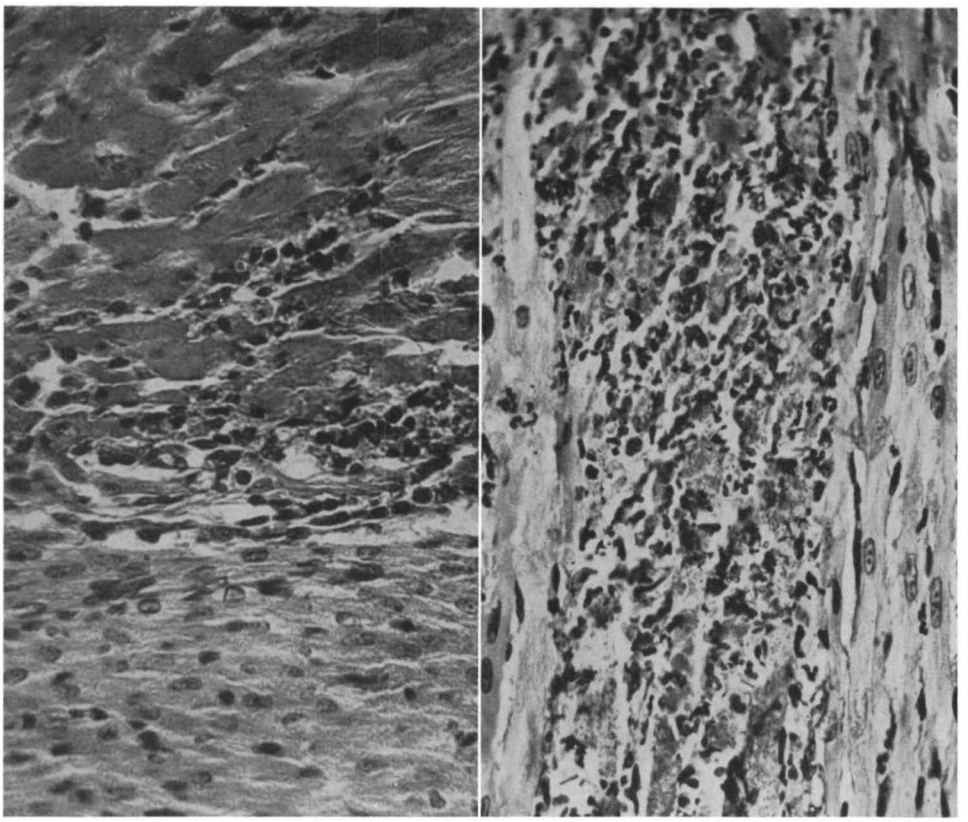

FIG. 2. Cardiac lesions in the heart of a rat treated with parathyroid hormone plus NaH_2PO_4. On the left, necrotic, homogeneous muscle fibres, which form an infarct-like region, sharply demarcated from the normal light-staining cardiac muscle. On the right, somewhat more advanced stage of the same kind of lesion. Here, the necrotic muscle bundle is heavily infiltrated by polymorphonuclear leukocytes. No calcification occurred in either of these foci (von Kossa's stain ×380).

croses and granulomas—similar to those seen in the "infarctoid cardiopathy" (2)—occurred without any sign of calcium deposition (Fig. 2). Apparently, necrosis may or may not precede calcium deposition, depending upon circumstances yet to be elucidated. In Groups III and IV, neither necroses nor calcium depositions were detectable in the myocardium or its vessels.

Nephrocalcinosis was of moderate intensity in Group I and severe in Group II. Most of the calcium deposition was found in the epithelial cells, as well as in the lumina of the tubules at the corticomedullary junction line (Fig. 3). Another, rather constant change in the animals of the first two groups was the development of intense perivascular edema around the larger arteries. On the other hand, nephrocalcinosis was absent in Group III and negligible in Group IV, although the periarterial edema was just as pronounced in these as in the first two groups.

In view of the virtual absence of cardiovascular and renal lesions, the

mortality in Groups III and IV was somewhat unexpected. However, these deaths should be ascribed, not to hyperparathyroidism, but rather to the inherent toxic effects of the comparatively large doses of $MgCl_2$ and KCl, because they occurred without any morphologic evidence of hyperparathyroidism, during the first 60 hours, at a time when all the rats of the first two groups were still alive.

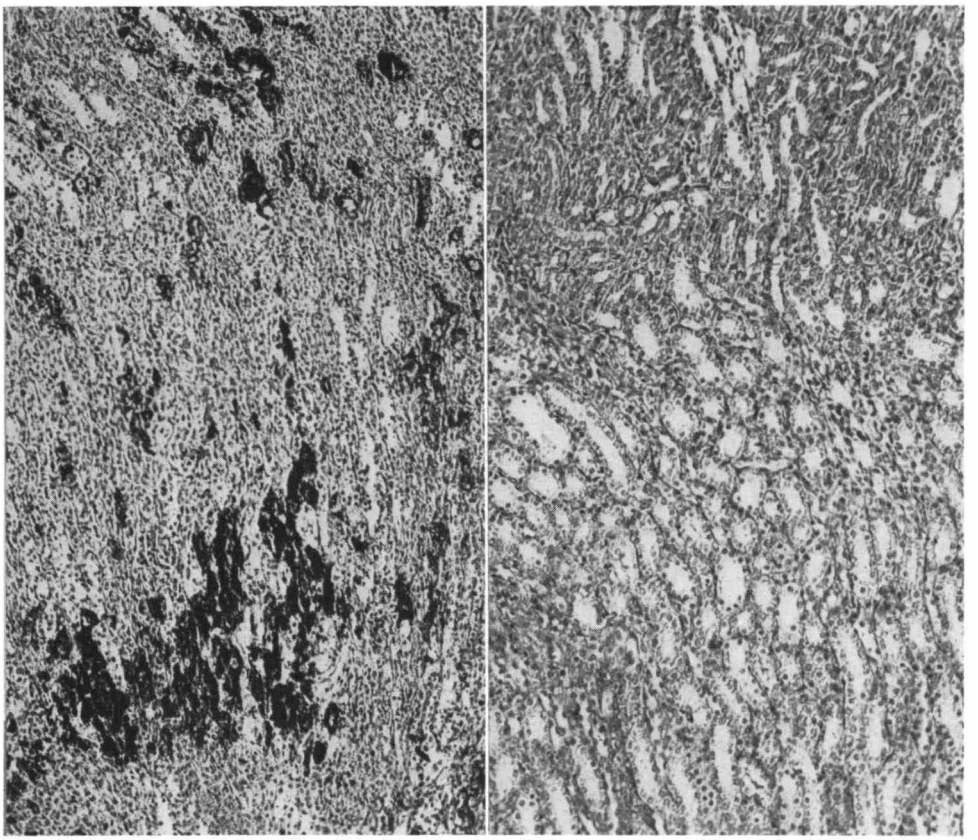

FIG. 3. Intense nephrocalcinosis in a rat treated with parathyroid hormone plus NaH_2PO_4 (left), in comparison with a corresponding region in the kidney of a rat which, in addition to parathyroid hormone and NaH_2PO_4, also received $MgCl_2$ (von Kossa's stain ×100).

Second Experiment

A more extensive experiment was then performed, in which parathyroid hormone was injected at the daily dose of 0.2 ml. twice daily, subcutaneously. NaH_2PO_4 and $MgCl_2$ were again given at the dose of 150 mg., in 2 ml. of water, twice daily, by stomach tube. Because earlier investigators have shown that it is possible to induce some degree of resistance to the toxic effects of potassium salts (5), KCl was administered in gradually increasing doses: 50 mg. during the first, 100 mg. during the second, and 150

mg. during the third and all subsequent days, always in 2 ml. of water, twice daily, by stomach tube. The experiment was terminated on the seventh day.

It is evident from Table 2 that the most intense osteitis fibrosa and soft-tissue calcification again occurred in the animals simultaneously treated with parathyroid hormone and NaH_2PO_4 (Group V). Additional treatment

TABLE 2. EFFECT OF ORAL TREATMENT WITH NaH_2PO_4, $MgCl_2$ AND KCl UPON PARATHYROID HORMONE INTOXICATION (SECOND EXPERIMENT)

Group	Treatment	Severity of lesions (Scale: 0–3)			Mortality (%)
		Bones	Heart	Kidney	
I	Ptr-H	1.0±0.2	0	0.1±0.1	0
II	NaH_2PO_4	0	0	0.1±0.1	0
III	$MgCl_2$	0	0	0	0
IV	KCl	0	0	0	30
V	Ptr-H+NaH_2PO_4	2.0±0.14	0.5±0.1	2.2±0.3	40
VI	Ptr-H+$MgCl_2$	0.2±0.14	0	0	80
VII	Ptr-H+KCl	0.1±0.1	0	0	60
VIII	Ptr-H+NaH_2PO_4+$MgCl_2$	0.7±0.2	0	0.1±0.1	0
IX	Ptr-H+NaH_2PO_4+KCl	0.5±0.25	0	1.2±0.2	20

with either $MgCl_2$ (Group VIII) or KCl (Group IX) exerted a prophylactic effect. However, contrary to out expectations, there was a very high mortality in the animals treated with parathyroid hormone plus $MgCl_2$ (Group VI), or parathyroid hormone plus KCl (Group VIII), without the concurrent administration of NaH_2PO_4, although the latter salt once again aggravated the usual effects (osteitis fibrosa, soft-tissue calcification) of parathyroid hormone intoxication. These observations suggested that NaH_2PO_4 in itself may antagonize the specific toxic effects of $MgCl_2$ and KCL. This supposition has been subsequently confirmed by another experimental series, in which these salts were given alone and in combination to animals that did not receive parathyroid hormone (to be published elsewhere).

In this second experiment (in which parathyroid hormone was given at a much lower dose level than in the first), the osteitis fibrosa was comparatively mild (Group I) and was intensely (and highly significantly) aggravated by concurrent treatment with NaH_2PO_4 (Group V), and ameliorated by $MgCl_2$ (Group VI) and KCl (Group VII). The particularly severe skeletal changes that occur when parathyroid hormone is administered in combination with NaH_2PO_4 (Group V) were likewise diminished both by $MgCl_2$ (Group VIII) and by KCl (Group IX).

In the animals treated with parathyroid hormone alone, or in combination with phosphate, the skeletal lesions were primarily characterized by intense osteoclastic bone absorption, while, in the rats concurrently treated with $MgCl_2$ or KCl, if skeletal changes were detectable at all, they usually

consisted of fibrous tissue proliferation and osteoblastic new-bone formation (Fig. 4). The latter type of response is very reminiscent of the early stages in the "marble-bone type" of proliferative reaction that is normally seen in rats treated with comparatively small doses of parathyroid hormone (6).

It is also noteworthy that the parathyroid hormone, when given without

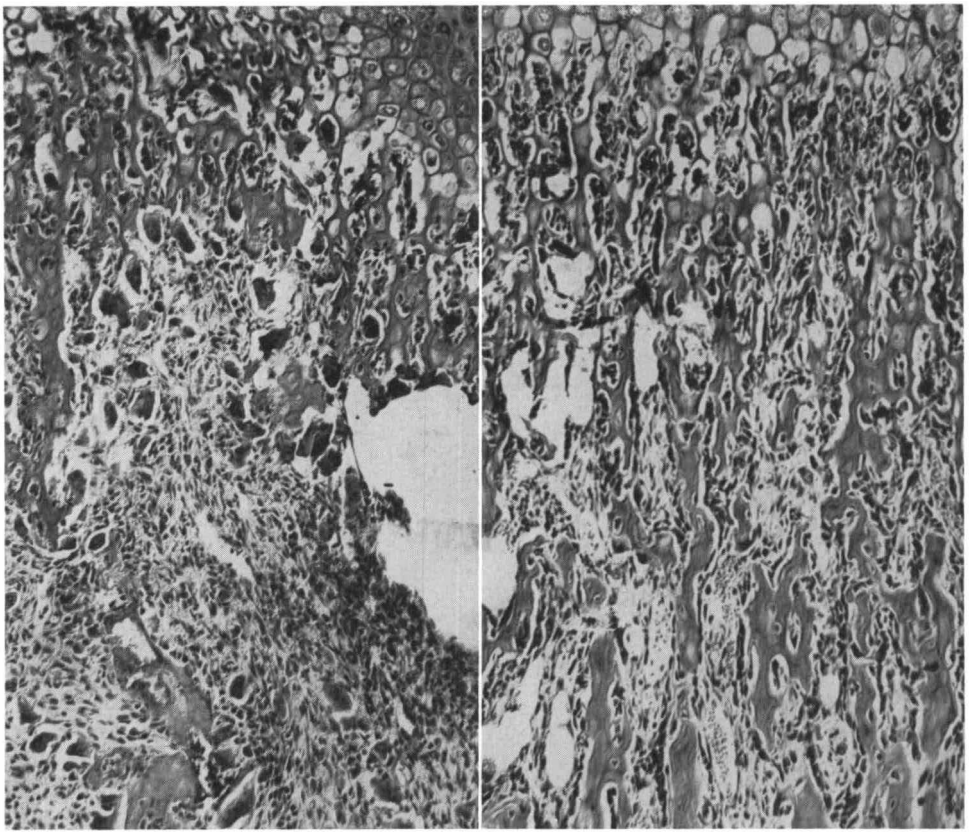

FIG. 4. Intense osteitis fibrosa, with numerous giant cells and cyst formation, in the region just underneath the distal growth-cartilage of the femur of a rat treated with parathyroid hormone plus NaH_2PO_4 (left), in comparison with the corresponding region in the femur of a rat given $MgCl_2$ in addition to parathyroid hormone plus NaH_2PO_4. Note that the magnesium salt did not completely abolish the effect of the parathyroid hormone, but greatly diminished osteoclast formation and bone absorption, although fibrous tissue and osteoblast proliferation are still evident (hematoxylin-phloxine ×100).

phosphate, but in combination with $MgCl_2$ (Group VI) or KCl (Group VII), induced depression, convulsions and pronounced hyperemia as well as edema of the brain. Such changes were not seen in the animals treated with parathyroid hormone (Group I), $MgCl_2$ (Group III), or KCl (Group IV), alone. Apparently, parathyroid hormone actually sensitizes the animal to

certain toxic effects of $MgCl_2$ and KCl, unless the specific toxic actions of these electrolytes are neutralized by the concurrent administration of NaH_2PO_4.

Third Experiment

The possibility still remained that the striking antagonism between phosphate on the one hand and magnesium or potassium salts on the other is merely due to some topical interaction between these electrolytes, which might interfere with the absorption of phosphate from the gastrointestinal tract. This question could be answered by experiments in which $MgCl_2$ or KCl would be administered in the usual manner, *per os*, while the phosphate would be given parenterally. However, it is impossible to administer the required large quantities of phosphate intravenously, subcutaneously or intraperitoneally. Hence, it was decided to introduce them through subcutaneous granuloma pouches. If a subcutaneous air-bubble is prepared under the dorsal skin of the rat and gradually increasing doses of irritants are introduced into the lumen of this cavity, the wall is soon transformed into a highly resistant granuloma pouch which protects the adjacent tissues from necrosis (7).

In view of this, a third experiment was performed in essentially the same manner as the second. However, in order to obtain more pronounced hormone overdosage effects, we administered the parathyroid extract at the dose of 0.2 ml. during the first three days and, after that, at the dose of 0.5 ml. At each level, the hormone was given twice daily, subcutaneously. $MgCl_2$ and KCl were both given at the dose of 50 mg. on the first day, 100 mg. on the second, and 150 mg. on the third and all subsequent days, always in 2 ml. of water, twice daily, by stomach tube. On the other hand, NaH_2PO_4 was administered through subcutaneous granuloma pouches. These were prepared by injecting 20 ml. of air under the shaved skin of the back, under ether anesthesia, and then introducing into the lumen of this cavity 25 mg. of a neutral mixture of NaH_2PO_4 and Na_2HPO_4 in 1 ml. of water. The same dose of this phosphate solution was injected into the pouches, without anesthesia, three more times during the first day. On the second day, 50 mg., on the third and all subsequent days, 75 mg. of the same neutral phosphate solution were injected four times daily into the granuloma pouches. The experiment was terminated on the seventh day (Table 3).

Given at this high dose level, the parathyroid hormone again produced a very intense osteitis fibrosa, about equal in its intensity to that seen in the first experiment; consequently the skeletal changes were less evidently aggravated (by NaH_2PO_4) or inhibited (by $MgCl_2$ or KCl) than in the second experiment, in which the osteitis fibrosa was milder. However, the results were otherwise essentially the same in this as in the previous two experimental series. Hence it may be concluded that the ability of $MgCl_2$ and

KCl to inhibit the intense hyperparathyroidism otherwise obtained by combined administration of parathyroid extract plus NaH_2PO_4 is not due merely to interference with phosphate absorption. Still, it is noteworthy that the magnesium and potassium salts are consistently and markedly effective only in preventing the cardiovascular and renal lesions of parathyroid hormone overdosage when the latter is aggravated by NaH_2PO_4.

TABLE 3. EFFECT OF NaH_2PO_4 (ADMINISTERED THROUGH GRANULOMA POUCH), $MgCl_2$ (ORALLY), AND KCl (ORALLY) UPON PARATHYROID HORMONE INTOXICATION (THIRD EXPERIMENT)

Group	Treatment	Severity of lesions (Scale: 0–3)			Mortality (%)
		Bones	Heart	Kidney	
I	Ptr-H	1.9±0.3	0	0.2±0.1	0
II	NaH_2PO_4	0	0	0.9±0.2	10
III	$MgCl_2$	0	0	0	0
IV	KCl	0	0	0	30
V	Ptr-H+NaH_2PO_4	3.0±0	1.6±0.3	2.6±0.25	50
VI	Ptr-H+$MgCl_2$	2.2±0.3	0	0.3±0.2	80
VII	Ptr-H+KCl	1.3±0.2	0	0	80
VIII	Ptr-H+NaH_2PO_4+$MgCl_2$	2.0±0.2	0	0	40
IX	Ptr-H+NaH_2PO_4+KCl	1.5±0.1	0	0	90

DISCUSSION

The striking inhibition of parathyroid hormone intoxication by magnesium and potassium salts that occurred under the experimental conditions described here is somewhat unexpected. However, several earlier observations suggested some relationship between the parathyroids and magnesium metabolism. It has long been known, for example, that the skeleton contains a very large portion of the total body magnesium (8, 9). Furthermore, hypomagnesemic tetany is a definite clinical syndrome, although parathyroidectomy does not appear to produce any constant changes in the blood magnesium level of experimental animals (10–12). Spontaneous hyperparathyroidism, as well as treatment with parathyroid extract, has been claimed to raise the blood magnesium (13, 14), but on this point the literature is quite contradictory (15). The fact that magnesium and potassium act essentially in the same manner in preventing the manifestations of the infarctoid cardiopathy (3, 16), of dihydrotachysterol arteriosclerosis (4), and—as shown by the present experimental series—of the phosphate-aggravated parathyroid hormone overdosage, is especially remarkable because, in several other respects, Mg and K ions are antagonists (17).

It is difficult to understand why, in these experiments, the magnesium and potassium salts markedly antagonized the parathyroid hormone intoxication only when the latter was aggravated by concurrent treatment with NaH_2PO_4. It is possible that when parathyroid hormone is given without NaH_2PO_4, the sensitization by the hormone to the toxic actions of

$MgCl_2$ and KCl (brain edema, convulsions, depression) predominates so much that the beneficial effects (upon the skeleton and soft tissue calcification) of these salts are overcompensated and a high mortality results. Hence, perhaps the milder degrees of hyperparathyroidism—induced in animals not sensitized to parathyroid hormone by concurrent treatment with phosphate—could be effectively combatted only with much smaller doses of magnesium and potassium salts. These points will be further investigated in future experiments, but in any event, the observations reported here show that, under certain experimental conditions, severe hyperparathyroidism can be effectively combatted by the oral administration of $MgCl_2$ or KCl.

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