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Provocation and Prevention of Potassium Deficiency by Various Ions.* (24113)

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Earlier work on the Electrolyte-Steroid-Cardiopathy that is characterized by massive, myocardial Necroses (ESCN) showed that, at least in the corticoid-conditioned rat, toxicity of sodium is decisively influenced by: (1) the anion to which Na is attached, (2) concurrent administration of various other cations. Thus, after sensitization with certain corticoids, Na_2HPO_4, NaH_2PO_4, $NaClO_4$ or Na_2SO_4 produced extensive myocardial necroses in rats, while equimolecular amounts of NaCl were ineffective in this respect. Furthermore, the ESCN elicited by corticoids plus sensitizing Na-salts was completely prevented by simultaneous administration of magnesium and potassium ions, particularly when these were given as chlorides. The nephro-calcinosis that normally accompanies the ESCN when the latter is produced with the aid of Na-phosphates was likewise prevented by KCl or $MgCl_2$(1). It has long been known, furthermore, that rats kept either on K-deficient(2, 3,4) or on Mg-deficient(3,5,6) diets also tend to develop focal myocardial necroses and nephrocalcinosis. In the case of Mg-deficiency, these changes are frequently accompanied by convulsions. Several observations suggested that an excess of Na can aggravate K-deficiency(7,8), but in none of these earlier studies were the effects of various Na-salts compared, because at that time there was no reason to suspect a dependence of Na-actions upon anions. The possibility of a replacement of K by Mg was also disregarded. Thus, for example, one group of workers claimed that administration of excess NaCl aggravates the myocardial necroses characteristic of severe K-deficiency(9,10). They arrived at this conclusion by substituting $MgCl_2$ for NaCl in their control rats. Schrader *et al.* (3) postulated that simultaneous Mg-deficiency does not significantly alter the course of K-deficiency in the rat; indeed, some workers believe that there exists an actual antagonism between Mg and K(11,12).

In view of our findings concerning participation of electrolytes in production of myocardial necroses by steroids, we wished to determine: (1) whether the cardiac necroses and other manifestations of dietary K-deficiency would be most markedly aggravated by those Na-salts that sensitize for production of the ESCN, and (2) whether the morbid changes usually ascribed to a lack of K are accentuated by a concurrent deficiency in Mg.

Materials and technics. In the first experiment, 60 female Sprague-Dawley rats, with a mean initial body-weight of 49 g (range 43-55 g), were placed on the "Low Potassium Diet" of the Nutritional Biochemicals Corp. (Cleveland, Ohio) for 7 days. This diet consists of: corn starch 64.2%; casein 30%;

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TABLE I. Provocation and Prevention of Potassium Deficiency by Various Ions (First Exp.).

Group	Treatment	Cardiac necrosis	Nephro-calcinosis	Muscular cramps	Mortality (%)
1	None	1.1 ± .30	0	0	10
2	Na_2HPO_4	2.7 ± .10	.3 ± .30	3.0 ± 0	100
3	KCl	0	0	0	10
4	KCl + Na_2HPO_4	.1 ± .10	.1 ± .10	Transient tremor	30
5	$MgCl_2$	.4 ± .20	0	0	30
6	$MgCl_2$ + Na_2HPO_4	1.1 ± .30	0	0	20

butterfat 3.5% calcium carbonate 1.3%; sodium chloride 1%; and a vitamin mixture which contains per 100 lb diet: Vit. A concentrate (200,000 units/g) 4.5 g; Vit. D concentrate (400,000 units/g) 0.25 g; alpha tocopherol 5.0 g; ascorbic acid 45.0 g; inositol 5.0 g; choline chloride 75.0 g; menadione 2.25 g; p-aminobenzoic acid 5.0 g; niacin 4.5 g; riboflavin 1.0 g; pyridoxine hydrochloride 1.0 g; thiamine hydrochloride 1.0 g; calcium pantothenate 3.0 g; biotin 20.0 g; folic acid 90.0 mg; Vit. B_{12} 1.35 mg. On 7th day, the rats were subdivided into 6 equal groups and treated as indicated in Table I. 0.5 mM of dibasic anhydrous sodium phosphate, Na_2HPO_4 (Baker), 0.25 mM of potassium chloride, KCl (Fisher) and 0.25 mM of magnesium chloride, $MgCl_2 \cdot 6H_2O$ (Merck)—all "Reagent" degree of purity—were administered in 2 ml of water, twice daily, by stomach tube. The experiment was terminated on the 13th day, that is, after 6 days of electrolyte treatment. In the second experiment, 50 female Sprague-Dawley rats, with a mean initial body-weight of 51 g (range: 43-55 g), were placed on the same "Low Potassium Diet" used in the first series. On the 7th day the rats were subdivided into 5 equal groups and treated as indicated in Table II. The treatment was the same as in the first experiment except that, here, 0.5 mM of Na_2HPO_4 was administered once daily, and 0.5 mM of KCl and of $MgCl_2$ was given twice daily by stomach tube, always in 2 ml of water. Furthermore, a special control group was added which received the same amount of Na, in the form of 1 mM of NaCl, as was given to the rats treated with Na_2HPO_4. This experiment was terminated on the 9th day, that is, after 2 days of electrolyte treatment. In both experimental series the heart and kidneys were fixed in neutral formalin and stained with the acid-fuchsin technic(1) for demonstration of early necrotic changes, and with von Kossa's silver nitrate technic for histochemical detection of calcium. Both cardiac necroses and the nephrocalcinosis have been assessed in terms of an arbitrary scale of 0-3, the means of these readings (with standard errors) being indicated in our Tables.

Results. The most striking change observed in the course of the *first experiment* was the development of intense muscular cramps and convulsions, in the Na_2HPO_4-treated rats (Group 2). There was at first a fine fibrillar tremor followed by slow spastic extensions of the hind paws, and, eventually, generalized convulsions with forceful extensor movements in both hind legs. These cramps began 2-3 hours after Na_2HPO_4 administration and resulted in 100% mortality within the first 24 hours. Slight transient tremor was also observed in the rats which, in addition to Na_2HPO_4, received KCl (Group 4) but in none of the other groups.

Yellowish patches of myocardial necroses were quite evident, even by mere macroscopic inspection, in most of the animals of Group 2. The histologic findings (Table I) indicate that after having been kept on the "Low Potassium Diet" for 13 days, even the otherwise untreated controls (Group 1) showed moderate cardiac necroses. These lesions were significantly aggravated by Na_2HPO_4 (Group 2), but prevented or diminished by KCl (Group 3) or $MgCl_2$ (Group 5). Indeed, even in the event of simultaneous treatment with Na_2HPO_4, both KCl (Group 4) and $MgCl_2$ (Group 6) exerted at least a partially protective effect upon the induction of cardiac necroses.

Nephrocalcinosis was negligible in all groups of this series, presumably because the animals most likely to develop it (those of

TABLE II. Provocation and Prevention of Potassium Deficiency by Various Ions (2nd Exp.).

Group	Treatment	Cardiac necrosis	Nephro-calcinosis	Muscular cramps	Mortality (%)
1	None	0	0	0	0
2	NaCl	0	0	0	0
3	Na_2HPO_4	2.5 ± .20	2.8 ± .10	3.0 ± 0	60
4	KCl + Na_2HPO_4	0	0	Transient tremor	0
5	$MgCl_2$ + Na_2HPO_4	0	0	0	0

Group 2) died within a few hours after the Na_2HPO_4 treatment. The detrimental effect of Na_2HPO_4 can definitely be ascribed to conditioning by the "Low Potassium Diet," because numerous control experiments(1) showed that, under similar circumstances, even fatal doses of Na_2HPO_4 produce no cardiac necroses or muscular cramps.

In the second experiment, Na_2HPO_4 treatment was reduced while the prophylactic administration of KCl and $MgCl_2$ was increased. This was done, in order to prolong survival and, thereby, permit development of morphologic changes in the rats treated with Na_2HPO_4 and, at the same time, to offer a better chance of protection by KCl and $MgCl_2$. The results summarized in Table II indicate that, under these conditions, marked cardiac necroses and nephrocalcinosis developed after 2 days of Na_2HPO_4 treatment following 9 days on the "Low Potassium Diet"; at this time the controls (Group 1) had not yet developed cardiac necroses. The experiment also shows that Na_2HPO_4 was not effective merely by virtue of its Na-content, since equivalent amounts of NaCl (Group 2) were completely ineffective. On the other hand, the somewhat larger doses of KCl and $MgCl_2$ given in this experiment offered virtually complete protection against the mortality as well as the cardiotoxic, nephrotoxic and muscular-cramp-producing effects of combined treatment with the "Low Potassium Diet" plus Na_2HPO_4.

Discussion. The earlier findings reported in our introduction created the impression that Mg-content of the diet is of little if any importance in determining course of K-deficiency(3). This is presumably why the Nutritional Biochemical Co. (Cleveland, Ohio), which prepares the "Low Potassium Diet"—that we, like so many other laboratories, have used—did not consider it necessary to check the Mg-content of this ration. We found it to contain 52 mg/kg, which is close to the minimal amount necessary for growth according to Tufts and Greenberg(13). On the other hand, the latter investigators claim to have demonstrated, by adding various concentrations of calcium phosphates to Mg-deficient diets, that "a high content of calcium in the diet increases the severity of magnesium deficiency." However, they disregarded the fact that when Ca was raised (from 0.39 to 1.66%), phosphorus was also considerably increased (from 0.45 to 1.00%). Although it is quite possible that Ca itself exerts a sensitizing effect, it is also evident from our experiments that PO_4 can do so even when not attached to Ca.

Summary and conclusions. When rats are kept on a "Low Potassium Diet" that contains only near minimal maintenance levels of Mg, there develop cardiac necroses which can be prevented both by KCl and by $MgCl_2$ administration. On the other hand, Na_2HPO_4 (unlike equivalent amounts of NaCl) rapidly provokes development of severe cardiac necroses, nephrocalcinosis and muscular cramps before this diet, in itself, produces any obvious morbid changes. The particularly severe K and/or Mg-deficiency syndrome induced by Na_2HPO_4– supplements, in animals on this diet, can be prevented by either KCl or $MgCl_2$. These observations highlight the importance of PO_4– and Mg-ions in the development of the syndrome usually ascribed to K-deficiency.

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Adenovirus Vaccine Evaluation Study in Naval Recruits.*† (24114)

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Acute respiratory disease in military inductees continues to be a major problem (1,2,3). During the past 4 years adenoviruses, particularly types 3, 4 and 7, caused 20 to 70% of acute respiratory tract infections among military recruits(3,4,5,6,7,8). Several studies employing polyvalent adenovirus vaccines for prevention of respiratory disease have been reported(9,10,11,12,13). In their first study, Hilleman and associates evaluated a formalin-inactivated monkey kidney culture vaccine composed of types 4 and 7 adenoviruses, prepared at Army Medical Center. A subsequent study was made by them of vaccine prepared commercially(13).‡ Bell and associates used commercially prepared§ kidney cell culture vaccine containing adenoviruses types 3, 4 and 7(12). All studies have shown these vaccines highly effective in preventing infections due to types 4 and 7 adenoviruses, the only prevalent types isolated from recruit populations.

The present report gives results of an adenovirus vaccine evaluation study among inductees at Naval Training Center at San Diego, Calif., where both types 4 and 7 adenovirus infections were prevalent in the population, from March 11 to July 3, 1957.

Materials and methods. Vaccine was prepared by Eli Lilly and Co. Seed adenoviruses, types 3, 4 and 7, obtained from Dr. Robert J. Huebner at N.I.H., where they had been isolated directly from humans in monkey kidney cell cultures. Pools of each virus type were grown in 16 ounce bottles of trypsinized monkey kidney cells. After filtration each pool was inactivated with formaldehyde (1:4000) at

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‡ Lederle Laboratories.

§ Parke, Davis and Co.