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Nutritional Factors in the Pathogenesis of Cardiac Necroses

Part I.

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With 8 figures in 23 details and 1 table

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Introduction

It has been well known for half a century that focal myocardial necroses, followed by inflammation, can occur in man, either as an apparently separate disease (FIEDLER's myocarditis) or as part of systemic infections (e. g., in diphtheria, typhus). A review of the literature has shown, furthermore, that cardiac glycosides (1), vitamin D derivatives (2), adrenaline (3, 4), K-deficiency (5), forced muscular exercise (6)—i. e., a number of apparently quite unrelated agents—can elicit essentially similar lesions in the myocardium of experimental animals. The principal structural characteristics (necrosis, calcification, inflammation) of these lesions, as well as their extent, position and speed of development, vary somewhat from case to case; but they have certain salient common features: (a) morphologically, they are characterized by focal necrosis with invasion of the damaged muscle tissue by inflammatory cells; (b) unlike the true cardiac infarcts, these necroses are not due to acute vascular obstruction, but presumably to biochemical changes in the myocardium. None of the earlier authors stressed these similarities. FIEDLER's myocarditis, the pharmacology of digitalis, the effects of adrenaline overdosage, the toxicology of vitamin D derivatives, the cardiovascular effects of muscular work and the essentiality of K as a nutrient are topics so far removed from each other that no investigator interested in any one of them could be expected to be well informed about the literature in all these fields.

Thus, many drugs and food constituents can produce focal myocardial necroses (7–9), but virtually nothing is known about the mechanism through which these agents affect the cardiac muscle. To gain fundamental information about the nature of any complex biological interaction, we have to develop techniques that permit us to analyze it by breaking it down into its constituent elementary units. By reevaluating the scattered literature concerning the experimental production of cardiac necroses we may conclude that most of the agents investigated and described elicit cardiac lesions only under certain circumstances. If this is true, it would mean actually that the organism possesses natural defensive mechanisms with which to defend itself against the potential cardiotoxic action of various agents. Furthermore, it would be possible to conclude that changes in the external and/or internal environment could significantly participate in the pathogenesis of necrotizing cardiopathies. Consequently, experiments were performed on homogenous animal material concerning the two following basic problems:

1. To what extent do systemic stress reactions participate in the development of various experimental cardiopathies? Does exposure to stress influence the potential cardiotoxic effect of other agents? Which are the factors that determine the susceptibility of the heart muscle to the pathogenic actions of stress?

2. Are there conditioning factors capable of selectively sensitizing or desensitizing the heart to the effect of various agents known to be partially cardiotoxic? Could agents become cardiotoxic under certain circumstances, which usually never produce cardiac necrosis even in lethal doses?

Let us merely point out here that for the purpose of these comparative studies, we employed as pathogens: (a) noradrenaline, vasopressin and thyroxin, as agents capable of producing "spotty myolysis" with disappearance of muscle fiber segments, without extensive necrosis or inflammation; (b) stressor agents such as forced restraint, quadriplegia, hot and cold baths, or surgical trauma, which likewise produce, but only occasionally, small necrotic foci (so-called "micronecroses"); (c) papain, because this proteolytic enzyme preparation elicits acute, miliary, disseminated myocardial necroses; (d) plasmocid (a rather toxic and now obsolete antimalarial), since it produces large, patchy myocardial necroses and myocarditic lesions; (e) dihydrotachysterol (DHT), a steroid of the vitamin-D group, because it causes intense calcium deposition in the heart and in the coronary arteries; (f) corticoids, such as methylchlorocortisol (Me-Cl-COL), fluorocortisol (F-COL), and desoxycorticosterone (DOC) plus Na_2HPO_4 (or NaH_2PO_4, Na_2SO_4 $NaClO_4$), since this combined treatment is highly effective in producing the typical electrolyte-steroid-cardiopathy (ESCN), characterized by massive, infarct-like cardiac necroses with secondary inflammation, but without any demonstrable histologic change in the structure of the coronary arteries.

In addition to those enumerated above, several other agents were also tried. Some (for example, Na-fluoroacetate, pentamethylentetrazol, Na-arsenate) were used particularly because of contradictory reports in the literature concerning their ability to produce cardiac necroses; others (for example, diisopropyl-fluorophosphate, *pseudomonas aeruginosa* infection), because they were not known to produce this type of cardiac damage. It was felt that comparative studies with such a wide variety of potentially cardiotoxic agents would give us some information concerning common features in the pathogenesis not only of cardiac necroses in general, but especially of those cardiac necroses that are not accompanied by acute occlusion of the coronory vessels.

Thus, experimental cardiopathies that are widely different in their histologic characteristics were studied in the albino rat. A part of this work will be discussed in the present paper, while many related data have already been summarized in earlier reviews (7–16).

One of the most interesting results of these investigations was the demonstration that the production of necrotizing cardiopathies by basically different means is uniformly aggravated by certain factors (i. e., corticoids, Na-salts, dietary K- and Mg-deficiencies, sudden stress situations, age and coronary sclerosis) and prevented by others (i. e., chlorides, dietary Na-deficiency, adaptation to stressor agents, hemodynamic changes and the condition produced by pregnancy). All these observations suggest that there

may be some common pathway in the mechanism through which various agents influence the heart muscle, thereby producing necroses and/or inflammation. However, our studies, at present, do not permit elaboration of a detailed biochemical theory of necrotizing cardiopathies. Nevertheless, it seems to be obvious that electrolyte shifts are important in the pathogenesis of cardiac necroses, and it may be that corticoids condition the cardiac cell by altering its affinity for certain ions—perhaps through changes in cell permeability.

An overdosage with Na-salts, administration of corticoids, exposure to stressors, and feeding with a low-K diet are all known to result in a positive Na- and negative K-balance. Consequently, it would be tempting to assume that the necrotizing cardiopathies produced by biochemical means are in all instances, hypokalemic. In this event, the Na-salts could act simply because of the well known antagonism between Na und K, and exposure to stressors merely by increasing mineralocorticoid activity. In fact, this reasoning is in keeping with the general hypothesis according to which the disturbance of the intra- and extracellular equilibrium of Na- and K-ions (more precisely, a continuous inflow of Na into the cell and the concomitant loss of K) would explain the pathogenic actions of stress and/or corticoids at the cellular level (9).

In the following pages a number of experimental findings and clinical observations will be summarized to show that, although it seems to be well established that hypokalemia is an important factor that sensitizes the heart to the potentially cardiotoxic actions of various agents, it is not possible to explain the pathogenesis of necrotizing cardiopathies by the over-simplified theory that some agents predispose to hypokalemia, and that the cardiac lesions precipitated by different means are merely the consequences of an aggravation of K-deficiency. We shall see that the Mg- and Cl-ions also play an active role in this regard, and it is most probable that we are dealing with complex ionic interactions.

I. The cardiopathy of K-deficiency

Production of cardiac necrosis by K-deficiency.—It has long been known that rats on K-deficient diets eventually develop a syndrome characterized by more or less extensive necroses (followed by cellular infiltration) in the myocardium and skeletal muscles; this is often accompanied by degenerative phenomena and sometimes by calcification in the kidney (5, 17–22). Essentially similar changes have been observed in pigs (22), mice (23) and dogs (21), kept on K-deficient diets.

It was at first claimed that these dietary myocardial necroses are actually due to a combination of K- and vitamin-B_6-deficiencies (22), but this contention has not been substantiated. Subsequent workers regularly succeeded in eliciting the cardiac changes with diets deficient only in K, not in vitamins. It has also been claimed that the cardiac and skeletal muscle necroses, normally produced by K-deficiency in the rat, can be prevented by simultaneous vitamin-B_1-deficiency (24). This finding is difficult to interpret, because vitamin-B_1-deficiency, in itself, tends to produce myocardial necroses. It is less unexpected that concurrent treatment with DOC (a mineralocorticoid known to cause

hypokalemia) further aggravates the cardiac lesions that normally develop in rats kept on K-deficient diets (25).

The literature on the production of myocardial necroses by various types of K-deficient diets has recently been the subject of a detailed and excellent review (26). Undoubtedly, dietary K-deficiency can cause severe myocardial damage in various species. For example, in a series of rats initially 3–4 weeks old, kept on a K-deficient diet from 4 to 327 days, macroscopically visible cardiac lesions appeared as early as the third week. Rather similar lesions were previously noted in animals kept on Mg-deficient diets; hence, Mg-supplements were administered to some of the K-deficient rats, but this had no influence upon the development of the cardiac changes. No search was made for possible myocardial calcification. The associated renal changes consisted of tubular degeneration, dilatation, and calcified cast formation (27), that is, changes now known to be characteristic of hypokalemia. In another series, weanling rats were maintained on a K-deficient diet for about 3 weeks. Miliary myocardial necroses developed, occasionally with mural thrombi in the auricles. The kidneys exhibited tubular casts and fraying of the epithelium of distal convoluted tubules. None of the organs were examined for possible calcification (28).

In summary we may say that in the K-deficient animals the earliest histologically detectable changes consist of swelling and loss of striations of muscle fibers. The fibers gradually become necrotic and finally disappear, leaving the containing sarcolemma. Associated with these changes there is an immigration of leukocytes into the area of necrosis. Our studies showed, furthermore, that usually there is considerable calcium deposition in the affected areas, but no calcium or hyaline deposition, in the coronary arteries. Electron-microscopic investigations revealed that prior to the development of necroses, K-deficiency produces swelling and disintegration of cardiac mitochondria (29). A deficit of body potassium is apparently essential for the production of these cardiac lesions. It is hardly surprising that SHCRADER and his colleagues (5)—who, in 1937, first made the highly important observation that in animals kept on K-deficient diets multiple foci of necrosis develop in the myocardium—englected to consider any etiologic relationship between the nutritional lesion that they had discovered and the necrotizing cardiopathies produced by other agents.

Potassium is the major intracellular cation, and, as such, plays a key role in the interrelationships between cell structure and function. It is, therefore not surprising that a deficit of this mineral is accompanied by alterations in cellular activity and anatomic integrity. Although—as it was already mentioned—there are multiple structural alterations in various organs (kidney, liver, skeletal muscle, etc.) of K-deficient animals, it is generally believed that the cardiac lesions are not secondary consequences of the functional and structural abnormalities observed in other organs.

Clinical observations.—Numerous clinical observations suggest that hypokalemia induced in various ways can also play a role in the development of diverse cardiopathies in man. For example, a patient with K-depletions due to chronic diarrhea died during the intravenous infusion of glucose (which certainly decreases blood potassium). Autopsy revealed focal myocardial necroses, myocarditis, and hydropic degeneration of the renal tubules—a

syndrome quite similar to that seen in animals kept on K-deficient diet (30). In an Addisonian treated with DOC and withdrawal of K from the diet, multiple minute foci of cardiac necrosis occured and were ascribed to hypokalemia (31). Two patients with diabetic acidosis exhibited focal myocardial necroses with infiltration by lymphocytes and connective tissue. Calcification was not mentioned, but the lesions were ascribed to hypokalemia (32). Two other patients with "cryptogenic hypokalemia" developed asthenia, painful muscular cramps, hypotension, nephrosis, and mild nephrosclerosis. In one, the skeletal muscle exhibited focal inflammatory lesions and disintegration of muscle fibers. There were also degenerative changes in the liver, but no mention was made of cardiac lesions (33).

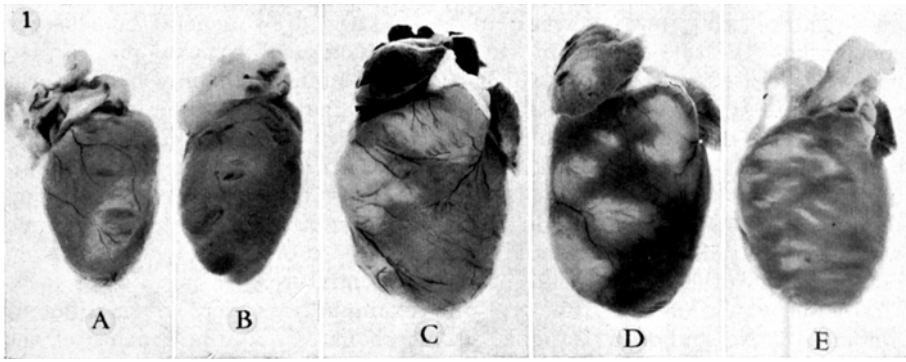

Fig. 1. *Macroscopic appearance of various types of cardiac necroses in the rat.* – *A*: The cardiopathy of K-deficiency; *B*: The cardiopathy of combined K-plus Mg-deficiency; *C*: Myocardial necroses following treatment with fluorocortisol plus Na-acetate while on a low-Cl diet; *D*: The electrolyte-steroid-cardiopathy induced by a combined administration of fluorocortisol plus Na_2HPO_4; *E*: Miliary, disseminated myocardial necroses produced by a single intravenous injection of papain extract.

In patients with hypokalemia caused by idiopathic steatorrhea or chronic ulcerative colitis, miliary cardiac necroses frequently develop in combination with the typical hypokalemic renal changes. It was especially emphasized that, in most of these cases, the coronary vessels remain normal or, at least, that arteriosclerosis is not in excess of what could be expected in patients of comparable age (34). In another patient with hypokalemia caused by the sprue syndrome, interstitial myocarditis with "clear-cell nephrosis" was demonstrable. The cardiac lesions consisted in necrosis of muscle fibers, with infiltration by polymorphonuclear leukocytes, lymphocytes and large mononuclear macrophages (35).

Many additional case reports of this type could be cited, but these will suffice to show that hypokalemia induced by various factors in man can elicit, not only arrhythmias and ECG-changes, but also structural alterations that are quite similar to the experimental cardiopathies discussed in the preceeding pages. Interestingly, none of the clinical observers envisaged possible connections between this type of myocardial lesion and FIEDLER's myocarditis, as well as the focal lesions induced in the heart by cardiac glycosides, adrenaline, forced muscular exercise, or corticoid overdosage.

Agents influencing the K-deficiency syndrome.—The development of the K-deficiency syndrome is largely dependent upon the simultaneous intake of

other *electrolytes*. In the K-depleted animals, Na tends to replace K in the composition of various organs, and particularly of the muscle (36–38). Hence, many attempts have been made to determine whether the cardiac and renal manifestations of K-depletion could be influenced by varying the Na-content of the diet.

It is difficult to interpret the results of the early experiments on the influence of Na upon the production of myocardial necroses by K-deficient diets, because the role of other anions and cations was invariably disregarded. Not until quite recently has it been recognized that the anion to which Na is attached exerts a decisive influence upon the activity of the latter and that the presence of other cations (particularly of Mg) can greatly influence the consequences of variations in the proportion of K to Na in the diet. Early investigators who were unaware of these facts drew general conclusions concerning "the role of sodium" in the production of hypokalemic cardiac necroses from experiments in which Na was administered only in the form of one of its salts, without controlling the participation of the anion. Indeed, sometimes, in diets designed to show the effects of simultaneous Na- and K-deprivation, Mg-salts were substituted for Na-salts (to keep the total mineral-intake constant). In fact, so little thought was given to the posible participation of other ions in the production of dietary myocardial necroses that some workers merely listed the Na- and K-concentration of the diet, without even recording the other constituents of their salt mixture.

In one experiment of this type, for example, rats were given different amounts of Na and/or K, supplied as bicarbonates, "at the expense of the ration". Here, the myocardial necroses became progressively more severe as the Na-content of K-deficient diets was raised (38). As we shall see, the effects of readily exchangeable, organic anions are very erratic.

In another investigation, "variations in either the potasium- or sodium-intake were made by adding known amounts of either cation to an otherwise basic diet". No mention was made of the form in which these cations were given nor of the incidence of cardiac lesions. Still, these observations led to the conclusion that myocardial necroses occur in rats kept on diets low in K and normal or high in Na (39). CANNON and his group (40, 41) claimed, furthermore, that the administration of excess NaCl aggravates the myocardial necroses characteristic of severe K-deficiency. However, these authors, unaware of the prophylactic effect of Mg-salts, substituted $MgCl_2$ for NaCl in their control rats. Therefore, it is very probable that, in this case, the aggravation of the myocardial necroses was unwittingly produced by the substitution of NaCl (a mild antinecrotic agent) for $MgCl_2$, which is—as our experiments clearly showed—a much more effective inhibitor of this type of cardiac necrosis.

It has also been observed that the production of myocardial necrosis by K-deficient diets can be prevented by simultaneously withdrawing the Na from the ration (39, 42). To explain this finding, it was assumed that only in the event of Na-deficiency is enough K retained by the tissues to prevent cardiac damage (39). Interestingly, if in such a doubly deficient diet the Na-content is normalized by the addition of $NaHCO_3$, the predisposition to cardiac necrisis is restored, while NaCl is ineffective in this respect (42). From this observation it was tempting to assume that changes in the pH of the diet play the decisive role, but more recent experiments indicate that both the anions

and the cations of various salts can significantly alter the K-deficiency syndrome quite independently of their effect upon tissue pH.

We found that when rats are kept on a virtually K-free diet that also contains only mere minimal maintenance levels on 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 the 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 also 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-deficieny (43)[1]).

$NaClO_4$ or Na_2SO_4, also produces cardiac necroses in rats maintained on the same diet; here again, both KCl and $MgCl_2$ exert a prophylactic action. However, unlike the phosphate, perchlorate, and sulfate, equimolecular amounts of NaCl fail to cause cardiac necroses (or muscular cramps), in rats maintained on this K-deficient ration (44). Here—as we shall see in many additional experiments—the Cl-anion itself appears to play a prophylactic role.

The most interesting outcome of all these investigations appears to us to be that Na-salts (Na_2HPO_4, $NaClO_4$, Na_2SO_4) previously shown to produce cardiac necroses in rats which had received corticoids (7) have the same effect in animals on a K-deficient diet. This similarity in the conditioning influence of K-deficiency and of corticoid overdosage is emphasized by the fact that equivalent amounts of Na given in the form of NaCl are ineffective in both these circumstances. Further, KCl and $MgCl_2$, which prevent the cardiac necroses produced by sensitizing Na-salts in corticoid-treated rats, exert this same protective effect in animals treated with sensitizing Na-salts while on a K-deficient diet. At least, in the case of the cardiopathy, the sensitization must be due to the Na, since all the salts of cations other then Na consistently proved

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[1]) For the details (materials, special techniques, composition of diets, etc.) of our studies discussed in this review, the readers will have to be referred to each original paper. Here—in order to make this synopsis easier to follow—we simply mention some basic points of our methodology. Unless otherwise specified, female SPRAGUE-DAWLEY rats, with a mean initial body weight of 50 gm (in all studies with synthetic diets) or 100 gm (in all studies on the normal laboratory diet, "Purina Fox Chow") were used. When the experimental animals were kept on a deficient-diet (e. g., K-, Mg-, or Cl-deficient ration) the controls always received a synthetic basic diet. In this manner, we hoped to eliminate any possible non-specific effect that might have been due to the pure synthetic diet. All experimental and control groups consisted of 10 animals. The diagnosis of cardiac necroses, nephrocalcinosis, and hepatic necroses was made with the aid of a dissecting loupe at autopsy. The diagnosis so obtained was checked in most instances with histologic sections. The severity of the lesions (necrosis, calcification, inflammation) was expressed in the form of an arbitrary scale of 0 to 3: 0 designating no lesion; 1 just detectable lesion; 2 moderate lesion; 3 marked lesion. It is important to note that sometimes the cardiac lesions (which are easily visible as yellowish or grayish patches between the darker healthy muscle tissue) can be graded more accurately by inspection of the whole heart then by studying a few histologic sections of each specimen.

ineffective in producing cardiac necroses after corticoid treatment (7); yet the anion also plays a decisive role, since NaCl was ineffective, both after corticoid-pretreatment and during K-deficiency. It is also noteworthy that, in the experimental series just summarized, even the animals which died with grade 3 cardiac necroses due to the Na_2SO_4 treatment never showed any trace of muscular cramps; hence, the effect of sensitizing Na-salts upon cardiac and skeletal muscle cannot be inseparably interrelated.

In order to compare the influence of *stress and of other potentially cardiotoxic agents* upon the hypokalemic cardiopathy, a large series of rats was kept on a K-deficient diet for a short time only so that no cardiac necroses would result from this pretreatment alone. A group of controls was otherwise untreated, while the remaining groups were exposed to threshold amounts of diverse potentially cardiotoxic agents. Additional control groups were kept on a basic diet, but exposed to the same mildly cardiotoxic factors.

Under these circumstances, severe cardiac necroses were produced by stressor agents (restraint, cold, heat, vagotomy, quadriplegia and traumatic injury), in the K-deficient but not in the control animals. Similar results were obtained on the same K-deficient diet with noradrenaline, vasopressin, thyroxin, DHT, and plasmocid; but since these compounds, in addition to producing nonspecific stress, also have specific cardiotoxic effects of their own, the relative importance of the two types of effects could not be determined. It was merely concluded that a brief period of nutritional K-deficiency can selectively condition the myocardium to the potentially cardiotoxic effect of various forms of stress (45).

It has been shown previously by FOLLIS (46) that, in rats maintained on a K-deficient diet, forced muscular exercise (swimming with a weight attached to the feet) increases the incidence of myocardial necrosis. At the same time other features of the K-deficiency syndrome were not aggravated; no morphologic changes were observed in the voluntary muscle. This is even more interesting, since the potassium content of the muscles of K-deficient animals is lowered and it would be expected that exercise, by causing a further reduction of this cation, might lead to necroses not only in the heart but also in voluntary muscles. The experiments of Follis indicate that the aggravation of the cardiac lesions by exercise cannot be explained so simply. Furthermore, since our experiments showed that all kinds of stressors (even those not associated with any increase in muscular work) can precipitate cardiac necroses in K-deficient rats, additional studies will be necessary to elucidate the mechanism involved.

Among other factors influencing K-deficiency, *lipids* are particularly important. In rats maintained on a K-deficient diet, the development of myocardial necrosis and fibrosis is accelerated if, at the same time, large amounts of corn oil are included in the diet (47). Subtotal *nephrectomy* also aggravates the cardiac lesions normally produced by K-deficient diets in the rat. The lesions so obtained were described as „focal necrosis and fibrosis" (48); calcification was not mentioned. It is well established that complete nephrectomy produces a predominantly calcifying cardiopathy accompanied by calcinosis of the coronary arteries (8, 49). Such vascular changes are not characteristic of the cardiac lesions induced by K-deficiency and further work will be needed to clarify the possible relationship between these two types of cardiac lesions.

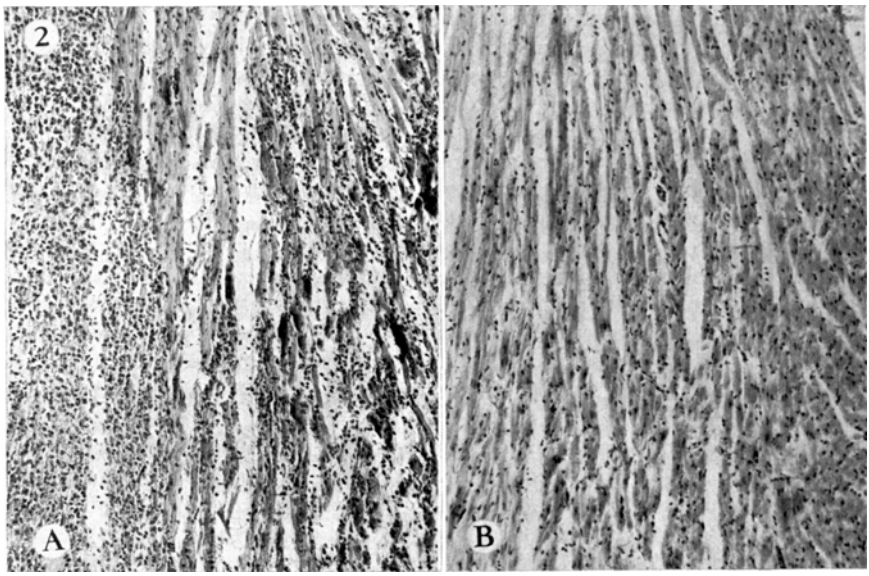

Fig. 2. *Sensitization by K-deficiency for the production of myocardial necrosis.* – *A*: Extensive necroses throughout the wall of the right ventricle, in a plasmocid-treated rat kept on a K-deficient diet. *B*: Absence of cardiac lesions in a similarly plasmocid-treated control animal allowed a normal intake of potassium (von KOSSA).

II. The cardiopathy of Mg-deficiency

Production of cardiac necrosis by Mg-deficiency.—In the first studies on the production of Mg-deficiency, it was noted that rats kept on a low-Mg diet exhibit, among other derangements, vasodilatation and changes in the rythm of the heart (50). Subsequently, cardiac changes (variously described as myocardial degeneration, calcification, fibrosis, necrosis, or inflammatory infiltration), sometimes with nephrocalcinosis and hepatic necrosis, have been noted repeatedly in rats (51–55), in rabbits (51), in dogs (56), and in cattle (57), on Mg-deficient diets.

A recent extensive re-examination of the pathologic changes induced by Mg-deficiency in the rat revealed them to be strikingly similar to those of K-deficiency. There are milairy necroses in the heart, nephrocalcinosis, and lesions in skeletal musculature. In addition, Mg-deficient rats develop a peculiar erythroderma (which is not seen in K-deficiency) and the number of mitochondria in liver and heart is significantly diminished. This latter finding was thought to play a role in the development of the gross histologic changes. It was noted, furthermore, that the electrochock threshold (EST) is increased on the Mg-deficient diet and can be further raised by adding cholesterol cholate or certain electrolytes to the diet. This shows how meaningless it is to speak simply of a „minimum daily requirement" of Mg, since the amount of Mg required depends upon other food constituents (58).

The usual procedure to induce Mg-deficiency in animals is to keep them on diets containing low amounts of Mg. Various formulations for such diets have been used (59–66) and the Mg-content in them varies from diet to diet and may range from almost – a traceas in the diet by KRUSE et al. (50) – to 120 parts

per million, as in the ration used by OSBORNE and MENDEL (67). Actually these latter two investigators made the first attempt ever, to investigate the effects of a Mg-deficient regime in mammals. Of the animals studied, the rat appears to have been the most common, followed by the calf, dog, chick, rabbit, mouse, hamster and crab, in that order. Although Mg-deficiency in man often has been studied as resulting spontaneously from various diseases, only more recently have there been some attempts made to induce the deficiency experimentally in human beings (68, 69).

It has been recognized by most workers using diets sufficiently poor in Mg that one of the constant consequences of Mg-deficiency is the animal's failure to grow fully. The younger the animal and the greater the degree of Mg-deficiency, the more marked the inhibition. In one of the studies it was observed that the littermate controls could be kept at the same weight as deficient rats by restricting their intake of Mg-containing food to 83% of the consumption of the deficient animals. This finding may be interpreted in the sense that on Mg-deficient diet—at least in the rat—there are increased fasting catabolism and decreased efficiency of utilization of energy and proteins (70). Apart from its influence on growth and body weight, the following manifestations of Mg-deficiency have been described and studied intensively: capillary dilatation and hyperemia as well as changes in the cardiovascular system in general; nervous excitability and convulsions; histologic alterations in the central nervous system; formation of cataract and development of exophthalmos; alterations in teeth and peridental tissues, changes in the gastro-intestinal system; metabolic alterations; formation of edema; changes in kidney, liver, bones and skin; and formation of stones in bladder (71).

In summary we may say that the ability of Mg-deficient regime to lead to cardiac necrosis is well established. However, some of the investigators claim that the incidence and severity of cardiac necrosis are very small in Mg-deficient rats. This may vary with the Mg content of the diet used, but may depend also upon other factors (experimental techniques, other constituents of the food, etc.). For example, in one experiment in which SPRAGUE-DAWLEY rats of an average initial body weight of 50 gm were restricted to the deficient diet for 12 days, the incidence of cardiac necrosis and nephrocalcinosis, as judged by naked eye examination, was 20% in either case (71). It was noted, however, that although the remaining hearts looked normal at the macroscopic examination, histologically most of them showed areas of necrosis of cardiac muscle fibers together with infiltration of lymphocytes and plasma cells as well as interstitial edema and tendency for capillary dilatation. These lesions are essentially similar to those seen in K-deficient animals and to those observed in the typical ESCN (induced by a combined administration of corticoids and sensitizing Nasalts).

Clinical observations.—Although the hypomagnesemic syndrome is well known in farm ruminants and in man, histologic alterations of the heart only occasionally were observed. In man, the Mg content of the blood and cardiac tissue is very variable in many forms of diseases, but not according to any regular pattern. Hypomagnesemia has been found under the following conditions (72): general illness, particularly postoperative states (with the prolonged use of Mg-free intravenous fluids), malignancy, diabetic acidosis, chronic renal disease, upper and lower nephron nephrosis, congestive heart failure undergoing therapy with NH_4Cl and mercuhydrin, epilepsy, eclampsia,

lupus erythematosus, hyperthyroidism, pancreatitis and, sometimes, ACTH administration. It is possible that the diminution of serum ultrafiltrable Mg may be a constant finding in the conditions, but in diabetes mellitus, pituitary and thyroid diseases, allergy, epilepsy and cancer, the Mg levels are inconstant and extremely variable (73). The recent finding of hypomagnesemia in a case with an excessive secretion of an aldosterone-like corticoid is interesting (74), as is hypomagnesemia in idiopathic calcinosis in infants (75). Secondary hypomagnesemia due to physiological or pathological derangements is also probably much more frequent (76, 77) than generally expected.

In regard to hypomagnesemia as a clinical disease, it may be said that the recognition of primary Mg-deficiency if it occurs, must possibly, but not inevitably, await the knowledge of forces that govern Mg in the body.

From a clinical point of view it is important to mention that Mg is a powerful vasodilator (78, 79). Even small amounts produce flushing and a sensation of warmth (80). In fact, this effect is constant enough to be utilized in determining the circulation time (81). Following the administration of Mg-salts in man, there is a slight initial bradycardiara than tachycardia (82). Since respiratory failure occurs only at 15 mEq per liter concentration (83), the toxic effects of Mg upon the heart are withouth any significance when therapeutic doses of Mg-salts are being dealt with. Notably, in coronary heart disease, the administration of Mg-salts (e. g., $MgSO_4$) have been claimed to be beneficial by several groups of workers (84, 85).

Agents influencing the Mg-deficiency syndrome.—The development of the Mg-deficiency syndrome—just as the occurence of the K-deficiency syndrome—is largely dependant upon the simultaneous intake of other *electrolytes*. For example, an increase in the Ca content of the diet aggravates Mg-deficiency and increases the body requirements of Mg (86). These findings were confirmed by others (87), and it was showed that the addition of high amounts of proteins to a high calcium—low Mg diet diminished the mortality rate, although, in itself, a high protein intake worsened Mg-deficiency. The same workers also investigated the opposite situation—the influence of low-Ca diet on Mg-deficiency. This too had an adverse effect and caused a decrease in the rate of growth, an increase in the mortality and the early appearance of the symptoms of Mg-deficiency.

The effects of high K intake on Mg-deficiency have also been studied. It was observed that additional K aggravates the growth inhibition of hypomagnesemia, and a combination of K and Ca was even more effective in this respect. Addition of Ca-salts alone led to increased mortality (88). The opposite condition, combined deficiency of K and Mg, was analyzed by SCHRADER et al. (5), in rats. They noticed the occurence of hyperirritability, but is was transitory and gave place to lethargy characteristic of K-deficiency. No skin lesions were noted and autopsy showed evidence of morphologic alterations characteristic of K-deficiency. Interestingly, in one experiment of TUFTS and GREENBERG (86)—studying the effects of a high Ca intake on Mg-deficiency—Ca was administered as the phosphate salt, but the authors did not attribute the worsening effect to phosphorus content. Moreover, it was concluded that the lesions are independent of the phosphate content of the diet.

As regards the cardiac changes, it was found that Na_2HPO (whether given orally or applied directly to the heart by iontophoresis) markedly increases the

incidence of cardiac necroses in the rat (58, 71). Other Na-salts, such as Na_2SO_4 and $NaClO_4$, also aggravate the Mg-deficiency syndrome (12), and, under certain experimental conditions, an interchangeability of K and Mg exists.

For example, in rats kept either on Mg—or on K-deficient diets for one week, the production of myocardial necroses by the intravenous injection of papain extract is greatly aggravated. This sensitizing effect of the K-deficient diet can be abolished by supplement of $MgCl_2$, and that of the Mg-deficient diet, by KCl (89).

In evaluating these data, it should be kept in mind that the K-deficient diet of the Nutritional Biochemical Corp.—that we, like so many other laboratories, have used—is also comparatively poor in Mg. We found it to contain 52 mg/kg of Mg, which is close to the minimal amount necessary for growth, according to TUFTS and GREENBERG (86). Hence, in the rats kept on this K-deficient diet, the $MgCl_2$ may have acted, in part, as a specific substituent for a relative Mg-deficiency that could have played a part in the sensitization to papain. On the other hand, the Mg-deficient diet contained ample amounts of KCl, yet, additional supplements of this salt were required to restore the heart's papain resistance towards normal. In view of these considerations, it is evident that the amounts of Mg and K required to maintain the structural integrity of cardiac muscle fibers under normal conditions do not provide optimal protection against papain; an excess of either KCl or $MgCl_2$ is nesessary to accomplish this. These observations confirm and extend our earlier finding, according to which KCl and $MgCl_2$ supplements augment the heart's resistance to the production of papain necroses in rats kept on the well-balanced "Purina Fox Chow" diet (90). It is particularly noteworthy, however, that in this respect, K and Mg can largely substitute for each other.

Additional experiments were then performed to obtain further information on the interchangeability of K and Mg in the production of myocardial necroses and the possible prophylactic effect of other chlorides. After sensitization with either a K- or a Mg-deficient diet, a short period of treatment with $NaClO_4$ suffices to cause wide-spread myocardial necroses of the ESCN-type in the vast majority of the experimental animals. On the other hand, this dietary conditioning to $NaClO_4$ is abolished, or at least markedly diminished, by concurrent administration of various chlorides. Only the prophylactic effect of NaCl was not significant during K-deficiency. It is of special interest that the sensitizing action of K-deficiency is decreased by $MgCl_2$, among other chlorides, and conversely, KCl protects against Mg-deficiency (12).

Under other experimental conditions, acute overdosage with $NaClO_4$ elicits a motor disturbance characterized by rather persistent, tonic extensor cramps, in the rat. Predominantly mineralocorticoid steroids – such as Me-Cl-COL and DOC – greatly facilitate these motor disturbances whereas glucocorticoids (e. g., cortisol and triamcinolone) prevent their occurence (91, 92). Furthermore, chronic treatment with Me-Cl-COL plus $NaClO_4$ produces a severe muscular degeneration resembling the juvenile type of muscular dystrophy; there is spotty myolysis and interstitial edema followed by inflammation around isolated necrotic fibers. These lesions in the skeletal musculature are usually accompanied by cardiac necrosis, and interestingly, both types of pathologic changes can be prevented by the administration of chlorides, especially KCl and $MgCl_2$ (93).

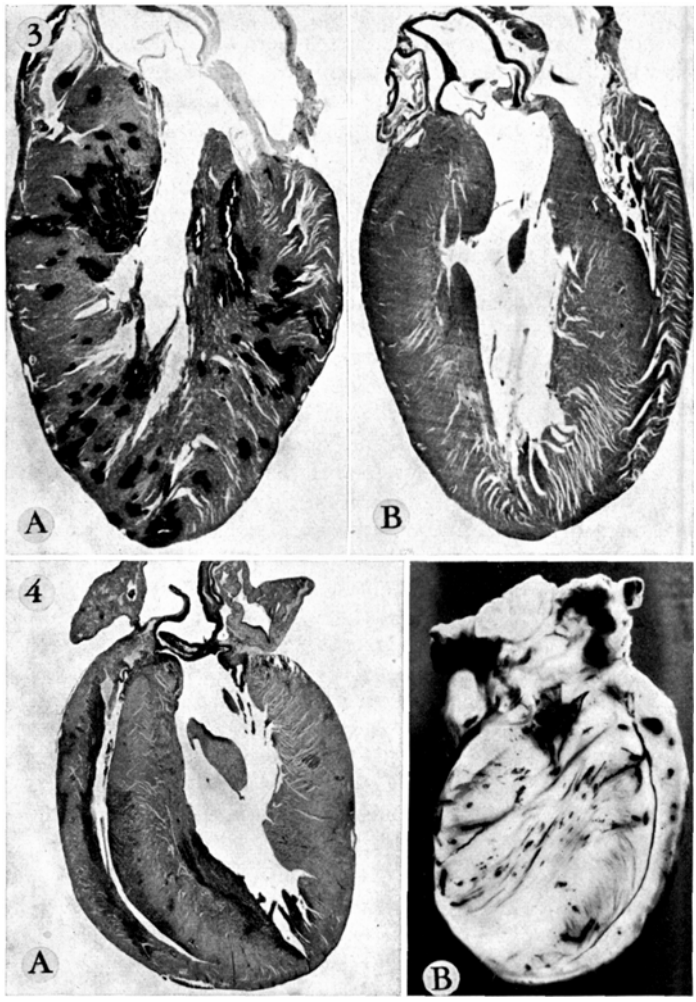

Fig. 3. *Sensitization by Mg-deficiency for the production of cardiac necroses by papain and prevention by KCl of the lesions normally so induced.* – *A*: General view of the myocardial necroses produced by papain in a Mg-deficient rat, in comparison (*B*:) with the heart of a similarly treated animal protected by a KCl supplement (Ref. 89).

Fig. 4. *Typical distribution of the lesions in the ESCN and as produced by DHT-overdosage.* – *A:* Cross section through the heart of a rat in which infarct-like, multiple massive myocardial necroses have been precipitated by neuromuscular effort after pretreatment with cortisol plus NaH_2PO_4. The necrotic and inflamed regions (here dark) show the preferential localization in the subendocardial layers (hematoxylin-phloxine). *B:* Distribution of the necrotizing and calcifying lesions (black area) in the heart of a rat treated with DHT (silver-impregnation). (Ref. 13, 133).

Another experimental series then indicated that – like Me-Cl-COL – a dietary deficiency in K or Mg predisposes the musculature to the toxic effects of $NaClO_4$. In such animals, the administration of perchlorate elicits particularly severe motor disturbances and marked degenerative changes in the skeletal muscles. In the early stage, these latter consisted of abnormally increased stainability, fragmentation, and loss of cross-striation of muscle fibers. The

more pronounced lesions were characterized by myolysis and interstitial edema; sometimes, inflammatory processes were seen around isolated necrotic fibers, often with infiltration by polymorphonuclear leukocytes and monocytes of the disintegrated areas (fig. 5). The occurence of all these pathologic manifestations was inhibited by the concurrent administration of various chlorides (e. g., KCl, $MgCl_2$, NaCl, NH_4Cl, $CaCl_2$) alone or in combination. In this respect, KCl and $MgCl_2$ were equally effective in both K- and Mg-deficient animals. Thus, the protective action exerted by the various chlorides is independent of the cations to which the chloride is attached (94).

It is interesting that in all these experiments the skeletal muscle lesions were modified by electrolytes in the same manner as the cardiac necroses that usually also develop under these experimental conditions and have been discussed earlier in this review. With respect to the pathogenesis of both types of lesion, it would be difficult to decide which among the various factors is actually the fundamental "primary pathogen" and which is the modifying factor responsible for the aggravation. Dietary deficiency of K or Mg can, in itself, result in similar muscle lesions if the rats are fed the diet for much longer periods than in the experiments just discussed (5, 18, 43, 54). On the other hand, long-term administration of $NaClO_4$ also produces muscular necroses, at least in corticoid-pretreated animals (9). Furthermore, both in man and in experimental animals, similar necroses can occur under the most diverse conditions (95). Presumably, this type of lesion is not characteristic of any one specific pathogen but represents, rather, a basic reaction of muscle to injury. Virtually, nothing is known of the factors that determine whether necrosis will affect skeletal and/or cardiac muscles in any given set of pathogenic circumstances. It is noteworthy, however, that under our experimental conditions, both the cardiac and the skeletal muscle necroses were prevented by the administration of various chlorides, and that KCl and $MgCl_2$ are equally effective in both, K- and Mg-deficient animals. It should be noted, furthermore, that similar protection was observed in experiments, using K- and Mg-deficient rats, when $NaClO_4$ was administered subcutaneously, while the various chlorides were given per os. This shows that the results obtained cannot be due to *in vitro* chemical reactions in the gavage mixture.

It was observed by MISHRA (58, 71) that, in the rat, various *stressors* markedly increase the incidence of Mg-deficiency cardiopathy. In his experiment, the stress of cold, heat or restraint, of a degree to which human beings may often be exposed, was applied to animals which were on Mg-deficient diet. These stressors, cold in particular, greatly aggravated the cardiotoxic effects of the diet used. Addition of a small Mg-supplement proved to be sufficient to restore the heart's resistance to normal.

Why stress should so dramatically aggravate the cardiovascular manifestations of Mg-deficiency cannot be easily accounted for. It is because stress by itself produces mitochondrial or any other abnormality which may be crucial for cardiac necrosis or is it because stress produces an increased K and/or Mg wash-out or their increased demand, so that stress synergizes with the cardiotoxic consequences of Mg-restriction? So far as Mg is concerned it may be mentioned that Mg excretion in urine is increased when rats acclimatized to ambient temperature are suddenly submitted to cold (96). In the absence of a balance-study this is difficult to evaluate, but if the increased Mg-diuresis

leads to a negative Mg-balance then this may be a possible cause of the aggravation observed. However, it is also known that cold leads to increased thyroid activity and output of thyroid hormones. This is obviously a protective

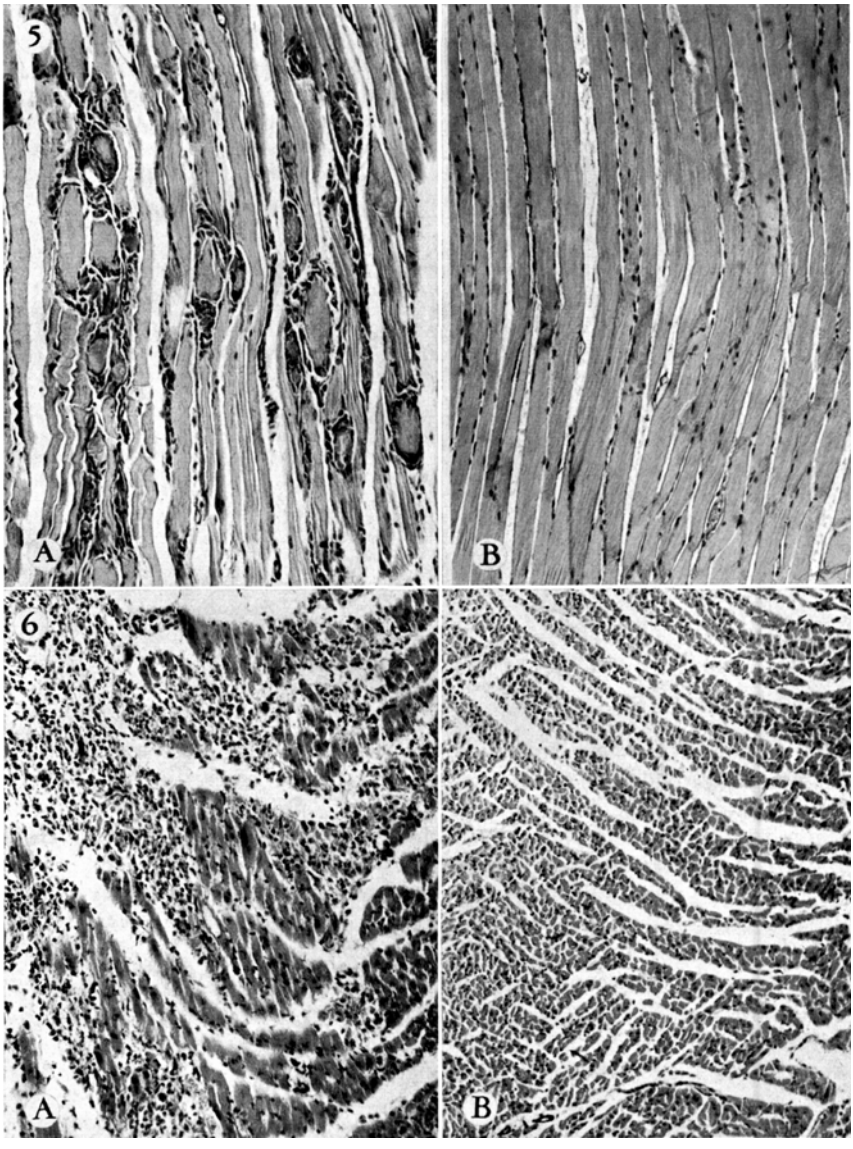

Fig. 5. *Sensitization by Mg-deficiency for the production of skeletal muscle lesions by perchlorate.* – *A:* Fiber necroses with phagocytic processes in the triceps surae of a rat treated with $NaClO_4$, while sensitized with a Mg-deficient diet. *B:* The pronounced structural changes shown in figure 4 A are prevented by additional treatment with KCl (PAS). (Ref. 94).

Fig. 6. *Sensitization by low-Cl intake to necrotizing effect of papain.* – *A:* Extensive necroses in the wall of the right ventricle, in a papain-treated rat kept on a Cl-deficient diet. *B:* Absence of structural alterations in a similarly papain-treated control rat allowed a normal intake of chlorides (von Kossa). (Ref. 105).

phenomenon and is liable to divert more energy into the production of heat. Thyroid hormone, in addition to its Mg-deficiency-like uncoupling of oxidative phosphorylation, also causes Mg-diuresis (97). This may then possibly be a second factor for the aggravation of cardiac necroses. Corticoids, too, are released during stressful situations. The role of these is not very clear, but is was reported that aldosterone increases Mg-diuresis (74), Since corticosterone is the major corticoid in the rat, it is likely that increased adrenal activity in this species may lead to Mg-diuresis. The worsening of cardionecrotic phenomena by stress due to heat or immobilization are even more difficult to understand in the absence of data concerning total Mg-balance in these conditions. In the case of restraint, excessive phosphate released by muscular contractions may be a factor. The protective role of Mg in another type of muscular stress, tourniquet shock, has also been demonstrated (98). It is interesting, furthermore, that necroses due to anoxic stress like that of K- or Mg-deficiency, are preceded by swelling and disintegration of cardiac mitochondria (99).

In connection with the effect of Mg upon the cardiovascular system, it is especially interesting that in rats (a species notoriously resistant to the production of atheromatosis), subintimal, sudanophilic, lipid depositions can be produced in the aorta and the cardiac valves by feeding a high *cholesterol*, low-Mg diet. There appears to be an antagonistic interaction between Mg and cholesterol. On diets containing threshold amounts of Mg, dietary cholesterol supplements precipitate the manifestations of Mg-deficiency (hyperexcitability, hyperemia of the ears, nephrocalcinosis, hypomagnesemia), while excess administration of Mg prevents the production of atheromatosis by cholesterol (100–102). Under these experimental conditions, the administration of thyroxin lowered the serum cholesterol values, abolished the kidney lesions and reduced the sudanophilia, even though serum Mg levels remained low (65).

In connection with this latter finding, we note that the administration of *thyroid hormone* can sometimes produce structural lesions in the heart (45) but the mechanism involved is not yet known. It is interesting, however, that, in young, growing rats, thyroxin-feeding increases the Mg-requirements. At the same time, the oxidative-phosphorylation efficiency of cardiac mitochondria is decreased and there develop typical signs of Mg-deficiency, which can be prevented by dietary supplements of Mg (103). Following Mg-deprivation, the oxidative phosphorylation of cardiac mitochondria is also rapidly impaired (104). Thus, the metabolism of cardiac tissue seems to be peculiarly sensitive to Mg-deprivation and to thyroxin. It is possible that the protection offered by $MgCl_2$ and KCl against various types of experimental cardiac necroses depends upon an inhibition of some metabolic defect related to this impairment of oxidative phosphorylation.

III. The cardiopathy of Cl deficiency

Cardiac muscle and variations in Cl-intake.—The briefly summarized previous investigations clearly showed that rats kept on a K- or Mg-deficient diet for a short period become sensitive to the potentially cardiotoxic effect of stress as well as that of many, otherwise unrelated, agents. It was soon observed that a lowered dietary chloride intake exerts a similar sensitizing effect on the myocardium. From these facts, it may be concluded that both the cation

(K^+ and Mg^{++}) and the anion (Cl^-) play an important role in the yet unclarified biochemical processes that sensitize or desensitize the myocardium against diverse potentially cardiotoxic agents.

The participation of the Cl-ion in the pathogensis of necrotizing cardiopathies was studied by varying the dietary intake of chlorides. Rats kept on low- or high-chloride diet, respectively, were subsequently treated with diverse cardiotoxic agents. A number of cardiac lesions were investigated, which differ widely from each other in both their causative agents and their histologic characteristics.

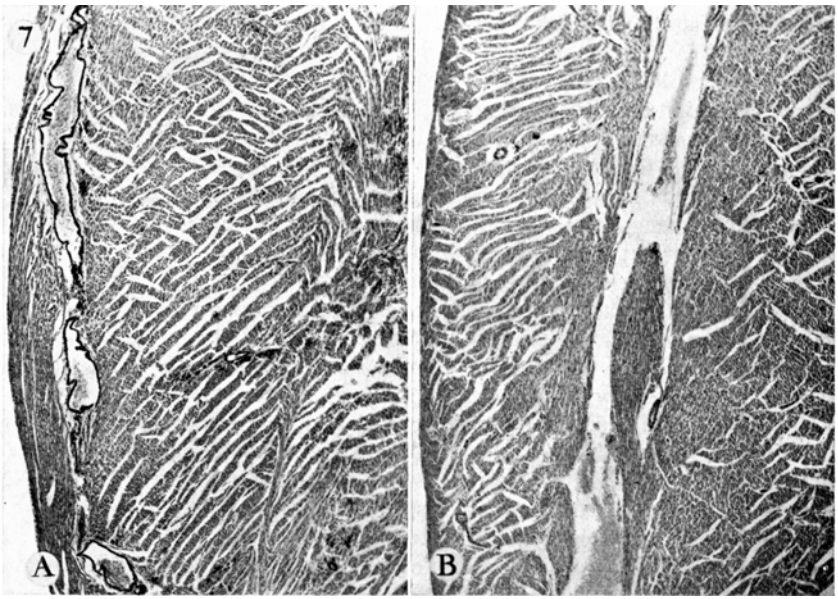

Fig. 7. *Prevention by high-Cl diet of cardiac calcification after DHT treatment. – A:* Severe calcifying lesions produced by DHT in a rat kept on a (synthetic) basic diet. *B:* Absence of calcification and necrosis in heart of a rat kept on high-Cl diet and given the same doses of DHT (von KOSSA). (Ref. 105).

It was found that Cl-deficiency significantly enhances the susceptibility of the heart to the production of necrosis, inflammation, and/or calcification by plasmocid, papain, DHT, Ca-acetate plus DHT, Na_2HPO_4 plus F-COL, Na-acetate plus F-COL, as well as by the stressor effect of forced restraint or quadriplegia after conditioning with F-COL. On the other hand, a dietary excess of chlorides proved to be effective in protecting the myocardium against all the cardiotoxic agents enumerated above (105). Only the spotty myolysis normally due to the administration of noradrenaline or vasopressin remained uninfluenced by variations in the dietary intake of chlorides.

It is obvious from the observations summarized above that a short period of dietary Cl-deficiency sensitizes the heart to various potentially cardiotoxic agents, whereas dietary Cl-excess exerts an opposite action. Nothing is known as yet about the mechanism through which variations in the dietary intake of chlorides so uniformly influence (aggravate or prevent) the various types of necrotizing cardiopathies that formed the subject of our investigations.

We note that, although the diets used in these experiments were identical as regards the ratios and amounts of cations, the variations in their Cl content naturally necessitated changes in other anions. The total Cl content of the low-Cl diet was 0.035%, that of the basic was 0.935%, whereas the high-Cl diet contained 1.62% of this anion (as determined by General Biochemicals, Inc.). The Cl-deficient diet differed from the basic diet in that in the former the chloride was replaced by corresponding bicarbonates on a molar basis, so the total amount as well as the percentage of the cations remained unchanged. Therefore, since the bicarbonate is generally considered innocuous (106, 107), the noxius effect of this diet may be attributed to its deficiency in chlorides. On the other hand, the great increase in the intake of chlorides with no change in the ratio of cations resulted in a high-Cl diet relatively deficient in bicarbonate and inorganic sulfate. However, the uniform and dramatic preventive effect of this high Cl diet as compared with the marked opposite effect of Cl-deficiency suggests that the variations in this anion may be the most important factor responsible for the results observed. This assumption is further supported by earlier findings in which, among the various electrolytes administered, no salts of anions other than chlorides offered significant protective action in the ESCN or in other experimental cardiopathies (13–15).

The pathogenesis of the necrotizing cardiopathy normally produced with noradrenaline and vasopressin (changes to which we usually refer as „spotty myolysis") seems to be basically different from all other types of cardiac necroses studied, since only the latter were influenced by the administration of electrolytes, by dietary Na-deficiency (108), or by variations in dietary intake of chlorides. It is interesting in this connection that RAAB (109, 110) concluded from his experiments that a sudden liberation of catecholamines within the heart muscle fibers could be responsible for the development of the ESCN. By depleting the catecholamines with reserpine, he succeeded in protecting the myocardium against this type of necrosis. Perhaps noradrenaline and vasopressin act at the final stage of the biochemical chain-reaction that results in necroses, whereas all other agents (steroids and electrolytes) exert their influence at intermediate points. Yet it would be difficult to explain on this basis why pretreatment with KCl and $MgCl_2$ offers considerable protection against the myocardial necroses normally produced by intravenous administration of proteases (15, 90) and even against the acute infarcts that follow surgical occlusion of small coronary vessels (111).

It was also observed that the combined administration of F-COL plus certain Na-salts, such as $NaHCO_3$, Na-citrate, -glutamate, -succinate (ineffective under ordinary conditions) becomes highly effective in eliciting acute cardiac necrosis when the Cl-content of the diet is lowered from a basic 0.935% to 0.035% (112). Further studies will be necessary to elucidate the mechanism through which Cl-deficiency activates the cardiotoxicity of F-COL and/or certain Na-salts. It is significant, however, that the same electrolyte-steroid combination is similarly activated, even in the animals fed the normal laboratory ration (i. e., Purina Fox Chow or a synthetic diet with an adequate Cl content) during exposure to stressful stimuli (12, 113).

All these observations suggest that chlorides play an important role in the pathogenesis of many types of experimental cardiopathies produced by biochemical means. It remains to be established whether variations in the

intake of chlorides similarly influence the occurence of spontaneous necrotizing cardiopathies in man, especially those unaccompanied by acute obstruction of coronary vessels.

Table 1. The effect of various agents upon skeletal and cardiac muscle as influenced by variations in chloride intake

		Abnormalities		
		Normal diet	High chloride diet	Low chloride diet
	Skeletal Muscle			
1	Fluorocortisol	No[1])	Cramps	No
	Triamcinolone + Desoxycorticosterone	No	Cramps	No
2	Fluorocortisol + Na_2HPO_4	Cramps	Aggr.	Aggr.
	Na_2HPO_4	Cramps	Aggr.	Prev.
	$NaClO_4$	Cramps	Aggr.	Aggr.
3	Dihydrotachysterol + Ca-acetate	Calcification	Prev.	Aggr. + Tetany
	Cardiac Muscle			
1	Fluorocortisol	No	No	No
	Triamcinolone + Desoxycorticosterone	No	No	No
2	Fluorocortisol + Na_2HPO_4	Necroses	Prev.	Aggr.
	Na_2HPO_4	No	No	No
	$NaCl_4$	No	No	No
3	Dihydrotachysterol + Ca-acetate	Necroses + Calcification	Prev.	Aggr.
4	Dihydrotachysterol	Calcification	Prev.	Aggr.
	Plasmocid	Inflammation	Prev.	Aggr.
	Fluorocortisol + Na-acetate	No	No	Necroses
	Fluorocortisol + Na-acetate + Stress	Necroses	Prev.	Aggr.
	Papain	Necroses	Prev.	Aggr.

[1]) Explanation of the abbreviations used: No = no lesion; Aggr. = aggravated; Prev. = prevented.

Skeletal muscle and variations in Cl-intake.—Our investigations concerning the effects of variations in Cl-intake upon the function and pathology of skeletal muscle give a number of interesting observations. For example, it was noted that an otherwise ineffective oral dose of Na_2HPO_4 (0.25 mM/100 gm of body weight) produces intensive muscular cramps in the rat (generalized continuous tremor and occasionally fits of generalized convulsions) when the chloride content of the diet is either increased to 1.62% or decreased to 0.035%, from a basic 0.935%. In rats kept on high-Cl diet for 14 days, subcutaneous injection of F-COL produces a qualitatively different type of neuromuscular dysfunction, with only a slight tremor but rather persistent, tonic extensor cramps, predominantly in the hind legs. Furthermore, sensitization with the low-Cl diet not only significantly aggravates the calcium deposition in the

muscular tissue due to combined DHT plus Ca-acetate administration, but in rats so treated acute paralysis develops (115). Table 1 summarizes the pathologic manifestations elicited by various agents, as influenced by variations in dietary Cl-intake.

The importance of chlorides for the maintenance of normal muscular function and structure is further supported by the additional observation that an electrolyte-steroid-induced, acute paralytic condition of the rabbit can also be prevented with various chlorides, especially by KCl or $MgCl_2$. More precisely, the severe paralytic condition normally induced by combined administration of certain steroids (Me-Cl-COL plus DHT) and NaH_2PO_4 was significantly inhibited by concurrent treatment by KCl or $MgCl_2$ (94). In the light of our previous studies, it was not unexpected to find that phosphate greatly aggravates the steroid-induced myopathy, whereas KCl prevents it. However, the similar protective effect of $MgCl_2$ is more difficult to understand. This is even more significant since the paralytic seizures produced with mineralocorticoid overdosage in animals closely resemble those occuring in familial periodic paralysis in man.

IV. Role of Na in the pathogenesis of cardiac necroses

Effects of Na-excess.—Our work on the ESCN showed that, at least in the corticoid-conditioned rat, the toxicity of Na is decisively influenced by: (a) the anion to which Na is attached, (b) concurrent administration of various other cations. Thus, after sensitization with certain corticoids, Na_2HPO_4, NaH_2PO_4, Na_2SO_4 or $NaClO_4$ produced extensive myocardial necroses in rats, whereas equimolecular amounts of NaCl were ineffective in this respect. Furthermore, as mentioned previously, the ESCN elicited by corticoids plus sensitizing Na-salts was completely prevented by the simultaneous administration of K- or Mg-ions, particularly when these were given as chlorides (7, 117–123).

It was found, furthermore, that certain Na-salts (phosphates, sulfates and perchlorate) can, whereas others (chloride, acetate, citrate, lactate, etc.) cannot, replace the stressors in eliciting myocardial necroses after corticoid conditioning. No salts of cations other than Na were effective, but these observations again show that the anion also plays an important role in the pathogenesis of this type of myocardial necrosis. We were particularly impressed by the fact that although NaCl, $NaHCO_3$ and the organic Na-salts were almost always totally ineffective after Me-Cl-COL pretreatment, they occasionally produced very pronounced cardiac necroses in rats damaged through fortuitous circumstances. A great number of various inorganic (NaCl, $NaHCO_3$, etc.) and organic (butyrate, formate, pyruvate, succinate, tartrate, etc.) Na-salts were then tested in combination with Me-Cl-COL and/or stressor agents, such as forced restraint. It was observed that these Na-salts are well tolerated, even by rats simultaneously treated with Me-Cl-COL or neuromuscular stressor. However, these same salts produce massive, and sometimes fatal, cardiac necroses in animals exposed to stress, after pretreatment with Me-Cl-COL. It was concluded, therefore, that during stress the metabolism of certain otherwise innocuous Na-salts and/or steroids is so altered that they acquire severe cardiotoxic properties (12, 113).

Let us recall here, that the same Na-salts (phosphates, sulfates and perchlorate) shown to produce an ESCN when administered together with corticoids, have the same effect in animals briefly sensitized by a K- or Mg-deficient diet. This similarity in the conditioning influence of dietary deficiencies and of corticoid overdosage is further emphasized by the fact that equivalent amounts of NaCl were ineffective in both these experimental conditions. However, following pretreatment with Me-Cl-COL plus DHT, severe cardiac lesions can be induced by many electrolytes, not only by the classical "sensitizing Na-salts". Under these conditions, the cardiotoxic effect cannot be ascribed to Na as such, since several Na-salts are inert in this respect, while others in which the cation is not Na (e. g., K_2HPO_4, Ca-acetate) exhibit severe necrotizing properties. Conversely, NaCl, which does not enhance the cardiotoxicity of either DHT or Me-Cl-COL, remains ineffective even when both these sterols are administered. Apparently, electrolytes sensitize the heart to the toxic effects of corticoids and of steroids of the vitamin-D group through some closely related mechanisms; consequently, these two types of steroids can potentiate each other's electrolyte-dependent actions (124–128).

It is notable, that not only the ESCN but the development of several other types of necrotizing cardiopathies (e. g., those normally produced by proteases, antibiotics, nephrectomy, gastric fistulas, digitoxin, or by the endocrine-kidney technique) are also significantly aggravated by the administration of Na-salts (9). All these observations suggest that Na plays an important key position in the pathogenesis of certain types of necrotizing cardiopathies that are induced by biochemical means.

Effects of Na-deficiency.—Experiments were then performed, in the rat, to examine the effect of Na-deficiency upon various experimental cardiac lesions, which differ widely from each other in both their causative agents and their histologic characteristics.

It was found that Na-deficiency significantly protected the heart against the production of necrosis, inflammation, and/or calcification by DHT, plasmocid and papain. In the F-COL-pretreated, Na-deficient animals the cardiac necrosis eliciting effect of neuromuscular effort was also markedly inhibited. A reduction of the severity and incidence of the "spotty myolysis" normally due to the administration of noradrenaline and vasopressin was suggestive, but not statistically significant (108).

It is obvious from these observations that dietary deficiency in Na-is very effective in desensitizing the heart against various potentially cardiotoxic agents. It is interesting that the pathogenic actions of those agents that were significantly synergized in previously discussed experiments by administration of certain Na-salts and/or corticoids were most markedly prevented by the Na-deficient diet. Furthermore, the observation that even the spotty myolytic changes caused by noradrenaline and vasopressin were somewhat reduced and the plasmocid necroses (which are so resistant to most other agents) were almost completely inhibited in the Na-deficient animals supports the assumption that Na plays an important role in the common biochemical derangement that produces necroses, inflammation and calcification of the heart muscle.

It is especially noteworthy that the Na-deficient animals were entirely protected against the intensive calcinotic effect of DHT. However, when

Ca acetate was administered even with smaller doses of DHT, this protective action of Na-deficiency was not observed. In the latter case the mortality was the same in the control and the deficient animals; all the hearts exhibited an acute, suppurative myocarditis with intensive calcification (MÖNCKEBERG type of sclerosis) of the coronary vessels. It is probable, however, that this resistance to therapy may have been due to the fact that the lesion was too severe to be prevented.

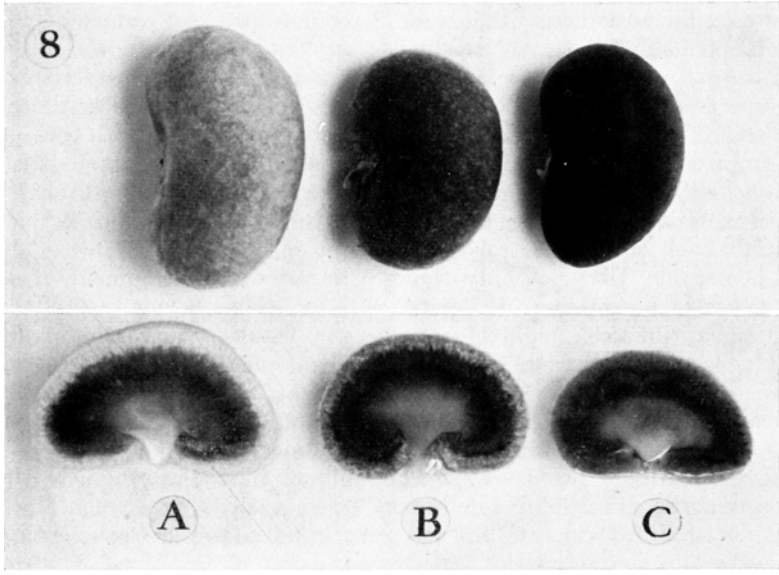

Fig. 8. *Prevention by Na-deficiency of the nephrocalcinosis normally produced by DHT-overdosage.* – *A* and *B:* Macroscopic appearance of cortical nephrocalcinosis produced with DHT and *C:* its prevention by Na-deficiency. (Ref. 108).

It remains to be established whether similar anion-cation interactions and, specifically, certain salt constituents of diets also play a role in the spontaneous arteriosclerotic and cardiac diseases of man.

(A brief summary and the literature quoted will be published at the end of Part II of this article.[1])

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[1]) Z. Ernährungswiss. **3**, No. 1 (1962, in proparation).