

The Effect On the Bones of Condensed Phosphate When Used as Food Additives : Its Importance in Relation to Preventive Medicine

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Abstract

Based on the fact that chemical products such as binding agents are produced by mixing three kinds of phosphates with different ratios, we mixed metaphosphate, polyphosphate and pyrophosphate. Each was made to Na-phosphate, K-phosphate, and Ca-phosphate and each was mixed with commercial feeds so that the content of P would be approximately 0.1, 0.15, 0.3, 0.4, 0.6 and 1.0%. The prepared pellets were given to ICR, CF # 1 and AKR strains of mice at 29 days of age for 680 days and observations were made through this experimental period at different stages. The observations were also carried out on the mice administered with the experimental feeds for 1.5 months from 9 to 10.5 months of age. The observations were compared with those of the control group at all times. As a result, plasma $1\alpha, 25(\text{OH})_2 \text{D}_3$ and P levels were always significantly higher in the phosphate administered groups relative to the control. Urine P and Fe increased while urine Ca decreased in the phosphate-treated groups.

The effect of phosphates on the bones was studied taking soft X-ray pictures of hind legs and applying microdensitometry to them. Through these observations we recognized thinning of the cortex of bones, reduction of marrow trabecules and development of osteophyte. Histological observations disclosed that changes in knee joint tissues were apparent; that is, a decrease in or an irregular loss of the number of cells in superficial, intermediate, and radial strata of the joint cartilage, proliferation of subchondral bone, and the development of osteophytes were noted. As for muscles, diameters of muscular fibers became smaller; in particular, type II fibers showed greater shrinkage. Regarding kidneys, swelling and atrophy of glomerular capillaries, proliferation of mesangial cells, nephrosclerosis, swelling, thinning, and loss of tubular epithelium, interstitial tissue inflammation, development of cylindruria, and deposition of calcium were observed. All these changes seem to be a particularly advanced aspect of the changes which are more pronounced with increasing dose and age.

These changes were found even in the group administered with the feed containing 0.1% phosphorus, and, these changes were dependent on the concentration level of P. It was observed that administration to older subjects for a short term (1.5 months) produced effects stronger than those to younger subjects administered for a long term (10.5 months).

The effects of condensed Ca-phosphate on bones were similar to those of condensed Na- and K-phosphates, and, hence, it was supposed that these effects were caused by phosphate radicals.

Key words : Food additive, Condensed phosphate, Bone, Osteoporosis, Kidney, $1\alpha, 25$ (OH) $_2$ D $_3$, Maximum non-effect level (MNL), Acceptable daily intake

Introduction

Condensed phosphate is a synthetic substance which is used most commonly as a food additive for various purposes and for various kinds of foods because of its diversified chemical properties.

Food additives were officially defined first in 1960¹⁾, and some of them were put into a class which had no required regulations of usage. Condensed phosphates have been categorized as food additives from the time of this official definition.

Recently condensed phosphates have been used for purposes other than as food additives as determined in the official book just mentioned; that is, they are added to fresh animal foods and vegetables and, since 1984, they have been used to remove the discoloration of supply water for buildings of assembled houses, offices, etc. or in the prevention of rust in the supply water pipe. Thus their fields of application have expanded.

Data obtained from the toxicity tests done to condensed phosphates when they were permitted as FA are not generally made known, but in the papers referred to in the handbook on "Japanese Standards of Food Additives" the following are reported: the acute toxicity test²⁾ and the subchronic toxicity test performed on cases in which 3% and 5% FA added feed was used showed renal calculi,³⁾ and 10%, caused an increase in renal weight, delay of growth and inflammation of uriniferous tubules;⁴⁾ there was little recovery of phosphate from urine⁵⁾; polyphosphate was confined to the cellular nucleus.⁶⁾

The relationship between Ca and the bone has been fully discussed. There are many reports on the balance of P and Ca in view of the nutritive elements, and their effects on the function of the parathyroid⁷⁾⁻⁹⁾. The effects of the parathyroid hormone on bones^{10) 11)} are discussed in many papers. However, among papers which deal with P, only a few observe its effects on the bones;¹²⁾ particularly those which observe the effects of condensed phosphates on the bones are very few. There are almost no studies which deal with P in natural foods and as additives observing its effects on bone tissues.

When condensed phosphates were first permitted as food additives, they must have passed the general toxicity test, but, as P has, together with Ca, activity toward the living body, we should examine particularly their effects on the living body which might occur because of their chemical characteristics. For the purpose of making such observations using sufficient and necessary indices, we proceeded with a series of studies first focusing on their effects on the bone.

The thinning of the bone cortex and the reduction of marrow trabeculas were most apparent in Na-phosphate-administered subjects,¹³⁾⁻¹⁵⁾ disarticulation of the femur and the tibia at the knee was larger in the K-phosphate group¹⁶⁾ and in the Ca-phosphate group¹⁷⁾ than in Na-phosphate group. Short term (1.5 months) administration to older subjects (9 months old) produced greater changes than longer (7 months) administration to younger subjects (29 days old).

Materials and Methods

Conditions of Animals and Foods

In view of the fact that three kinds of condensed phosphates are used in different mixing ratios for the purpose of binding agents,¹⁾ we mixed metaphosphate, polyphosphate, and pyrophosphate in the ratio of 7 : 1.5 : 1.5 and the mixture was added to commercial feed (CE-7, Japan CREA's product) so that P was in the feed at approximately 0.1, 0.15, 0.3, 0.4, 0.6, and 1.0% (2.5% was used when a mutagenicity test was carried out). The prepared pellets were given to ICR strain mice (some part of the experiment was made on CF # 1 and AKR strains). The number of animals included 10 mice in each group. From 29 days of age, they were fed with the pellets for 45 days or 680 days. During these days, on the 210th~240th days and the 450th day, various indices were examined. Also, observations were made on the subjects which were fed with phosphates for 1.5 months from 9 months of age to 10.5 months of age. Not only the subjects fed with condensed Na-phosphates but also those fed with K-phosphates were observed and each observation was compared with the control which was fed with non-phosphate-added feed given at the same time as the experimental subjects. Both phosphate-added feed and non-phosphate-added feed contained P and Ca which were derived from the original ingredients of the used commercial feed. The ratio of P to Ca was 0.98 : 1.0. Accordingly, the P level of condensed phosphate-added feed was about 1.1~2.0% and the ratio of P to Ca was 1.1~2 : 1. There were 10 mice in each group. The animals were reared in a room with a semi-barrier system at the Experimental Animal Center, Toho Univ, Sch. of Med., with a daily light cycle from 8 : 00 to 20 : 00.

1. Sister chromatid exchange

A study of the presence of mutagenicity through observations of frequency of sister chromatid exchange (SCE) in mouse lymphocyte chromosomes¹⁸⁾

Experimental subjects were :

- 1) ICR strain mice, ♂, 8 to 10 weeks old.
CE-7 added with mixed condensed Na-phosphates and each condensed Na-phosphate, 1%, 5%, and 10% (P contents, 0.25%, 1.25%, and 2.5%) was administered for 1 or 2 weeks.
- 2) ICR strain mice, ♂, 3 weeks old.
CE-7 added with three mixed condensed Na-phosphates, 1% and 5% (P contents, 0.25% and 1.25%) was administered for 20 months.

A lymphocyte from the abdominal cavity was cultured in the routine method. The SCE frequency of 20 cells from each subject was scored (in vivo). In addition, lymphocytes in the peripheral blood of a healthy human adult were cultured in the lymphocyte cultivation medium to which mixed condensed Na-phosphates and each of three condensed Na-phosphates were added in vitro at the final concentration of $1 \times 10^{-4}\%$ ~ $1 \times 10^{-1}\%$. SCE's in 30 cells of each subject were counted (in vi-

tro).

2. Bone Examination

Microdensitometric study was done on soft X-ray photographs of the femur and the tibia of the hind leg and the knee joint to measure bone cortex, marrow trabeculas, and bone width. Histological observations were done on the form of the knee joint, the knee joint cartilage, and the subchondral bone.

Hind leg bones were photographed with soft X-ray (SOFTEX-CSM, SOFTEX KK) and the films were examined. The densities of bone cortex and marrow trabeculas were measured microdensitometrically with the shadow at the intermediate position of the femur. The knee joint was fixed with 10% formalin, and, after decalcification, a specimen was made in the routine method. Then it was H.E. stained and observed with an optical microscope.

3. Measurement of various blood indices

Na, K, Cl, Ca, P, and $1\alpha, 25(\text{OH})_2\text{D}_3$ concentrations of blood, Na, K, Cl, Ca, P, and Fe concentrations of urine, and Ca and Fe concentrations of feces.

Samples of blood and urine were collected when a sacrifice was made. Feces samples were obtained during the week immediately before the sacrifice. Na, K, Cl, Ca, and P were measured in the routine method of clinical examination. Ca and Fe in feces were measured by atomic absorption analysis after wet ashing. $1\alpha, 25(\text{OH})_2\text{D}_3$ was measured by radio-receptor assay.

4. Histological observations of muscles

Observations of changes in the diameters of type I and type II fibers of the soleus in enzymatic histological method using ATP-ase stained specimens.

A frozen section of the soleus was ATP-ase stained and muscular fibers were measured according to types of fibers.

5. Histological Examinations

Changes in liver, kidney, and muscular tissues

Specimens of liver and kidney tissues were stained with H. E. and PAS, and then examined with an optical microscope. Specimens of soleus tissues were H.E. stained and observed with an optical microscope.

Statistics

Statistical analysis of these experimental results were done with the Student's t test or the Mann-Whitney U test.

Results

1. Survival ratio

No deaths were observed in the experimental period.

2. SCE

Observations of chromosomes of lymphocytes in the abdominal cavity of the mice fed with condensed Na-phosphates (*in vivo*) and in the cases in which condensed phosphates were added to the cultivation medium for lymphocytes in peripheral blood (*in vitro*) were compared with the control group. Neither observation showed a different SCE frequency from the control.

The result of the mutagenicity test based upon changes in the SCE frequency was negative.¹³⁾

3. Bone examination

Soft X-ray photographs of the femur, the tibia, and the knee joint showed thinning of the cortex, reduction of marrow trabeculas, disarticulation of the femur and of the tibia at the knee, and development of osteophytes. When the feed had 0.5% or 1% condensed phosphate (P content of the feed was approximately 0.1% and 0.2%) these changes were found following more than one year of feed administration, and in the case of 2% or 5% phosphate (P, approximately 0.4 or 1%), upon a 6-month administration. (Fig.1)

A decrease in the bone mass of the cortex and the marrow trabecula was made apparent through a densitometrical study of soft X-ray photographs.(Fig. 2, 3)

Through histological observations of the knee joint, superficial, intermediate, and radial strata of cartilage disclosed a reduction of their cell numbers, a disorderly arrangement of cells, and desquamation of each stratum. Also proliferation of the subchondral bone, development of osteophytes, and a deformity of the knee joint were observed. (Fig.4,5)

4. Measurement of various blood indices

1) Pellets with 0.5% and 2% mixed condensed Na-phosphates (P content of the pellet, approximately 0.1% and 0.4%) were given to ICR strain 29-day-old mice for about 650 days and observations were made when the mice were 680 days old. The findings were: The group fed with the pellets having 0.4% P showed a significantly elevated level of blood P compared with the control and 0.1% P groups; a significantly high level of urine Na was shown in the group administered with the feed having 0.4% P; urine P increased significantly in both the 0.1% P and 0.4% P groups; the level of urine Cl was significantly low in the 0.1% P and 0.4% P groups; urine Fe level was significantly low in the 0.4% P group; a significantly decreased level of urine Ca was seen in the 0.4% P group; the level of feces Fe showed an increasing tendency, though there were not statistically significant changes; no change was noticed in the case of feces Ca.¹⁴⁾

2) Pellets which contained 5% mixed condensed Na-phosphates (about 1% P) were given to the CF # 1 strain mice. A group of mice was fed with this experimental feed to the 29 day old mice for 6 months (young, medium group). The other group (older, short group) started eating the experimental feed at the age of 9 months and continued for 1.5 months.

The results of the observations of indices of their blood were: P and $1\alpha, 25(\text{OH})_2\text{D}_3$ were elevated very much in both groups, Na was elevated only in the 'older short group', and Ca, K, and Cl did not change. (Fig.6)

3) Pellets which contained 5% and 1% mixed condensed K-phosphates (about 1% P and 0.2% P) were given to the CF # 1 strain mice of 29 days. One group was fed for 6 months and the other group for 12 months. The results of the observations disclosed that in both groups blood P, K, and $1\alpha, 25(\text{OH})_2\text{D}_3$ were significantly increased. (Fig.7)

4) Pellets containing 5% mixed condensed Ca-phosphates (P, approximately 1%) were given to the CF # 1 strain mice which was 29 days old for 12 months and pellets mixed with 1% and 2% of the same phosphates as above (P, approximately 0.2% and 0.4%) were given for 18 months starting on the 29th day after birth. These groups did not show changes in Na, K, and Cl, while P and $1\alpha, 25(\text{OH})_2\text{D}_3$ significantly increased in both

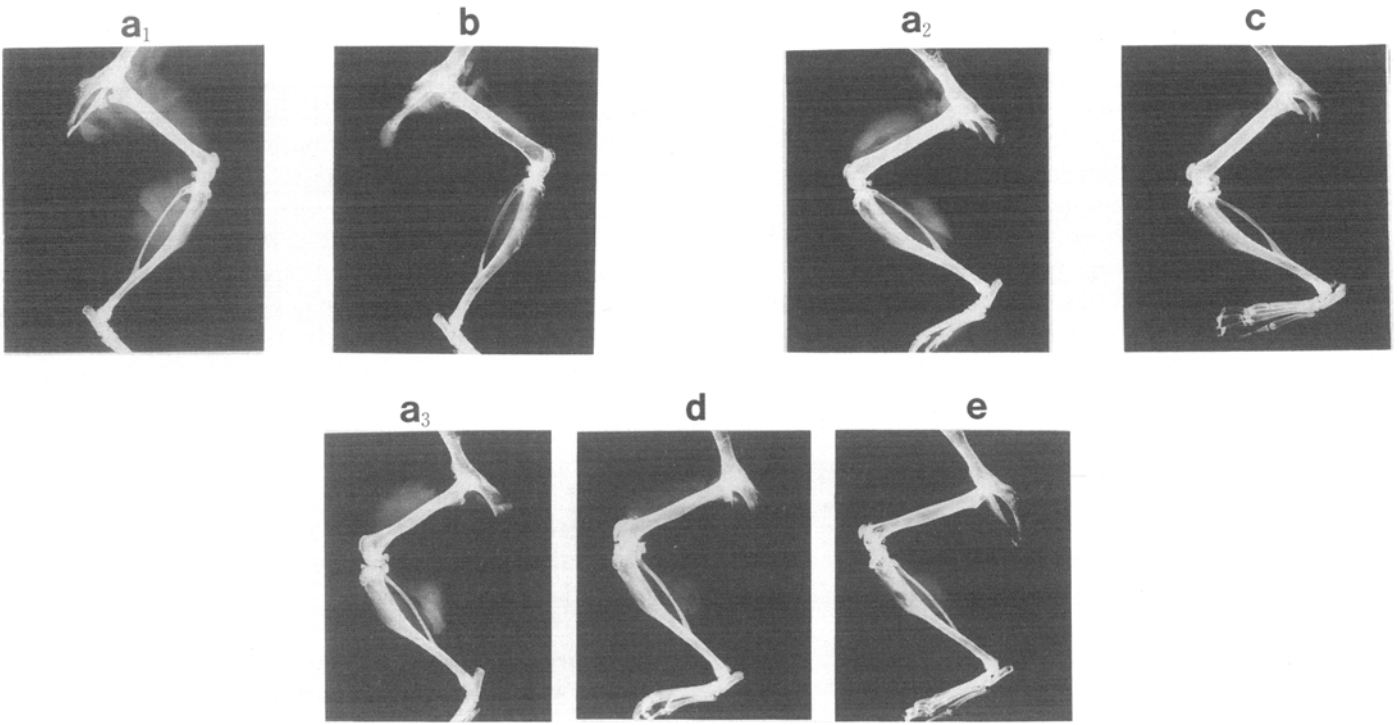


Fig. 1 Soft-X-ray findings of hind leg bones of mice.

Control subjects at 19 months of age (a₁, a₂, a₃). Treated subjects were fed 1% mixed sodium condensed phosphates (0.2% phosphorus feed) (b), 1% mixed potassium condensed phosphates (0.2%-P-feed) (c), 1% mixed calcium condensed phosphates (0.2%-P-feed) (d), and 2% mixed calcium condensed phosphates (0.4%-P-feed) (e) for 18 months. The figures disclosed thinning of the cortex, reduction of marrow trabeculas, disarticulation of the femur and the tibia at the knee, and development of osteophytes.

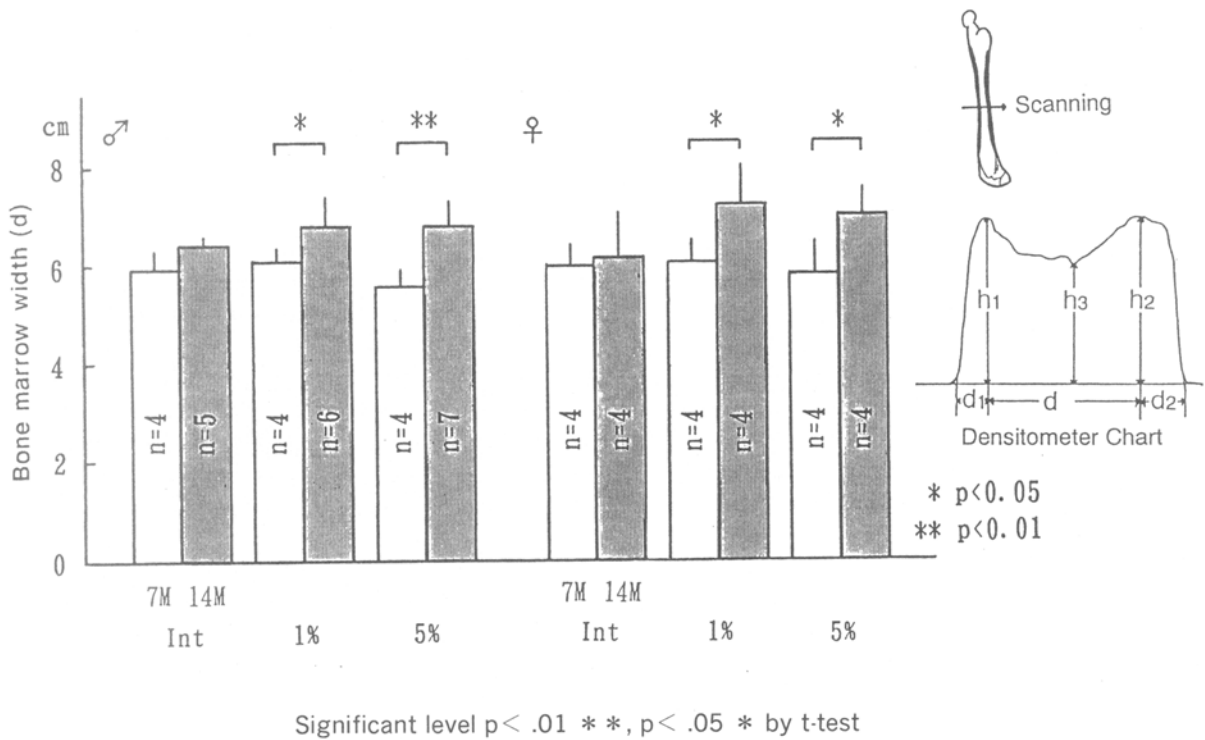


Fig. 2 Effects of mixed sodium condensed phosphates on the femoral bone marrow width (d) of mice by age.

Int. : Control groups at 7 months of age (7M), and at 14 months (14M).

1%, 5% : 1%, 5% mixed sodium condensed phosphate (0.2%, 1% phosphorus) added pellet for 7M and 14M were fed to the groups.

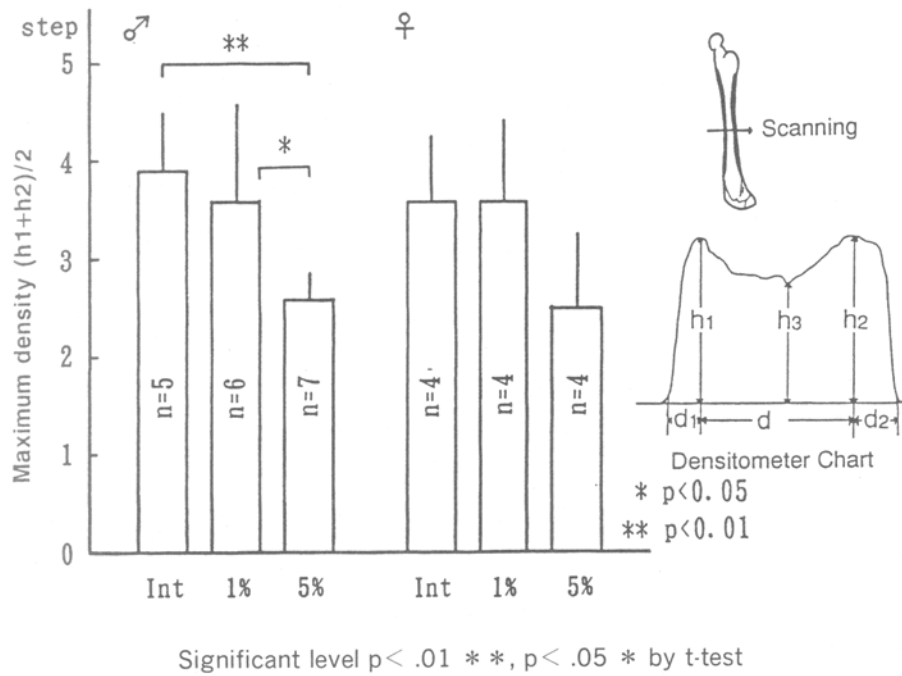


Fig. 3 Effects of mixed sodium condensed phosphates on the maximum density of the femoral bone cortex $(h_1+h_2)/2$.

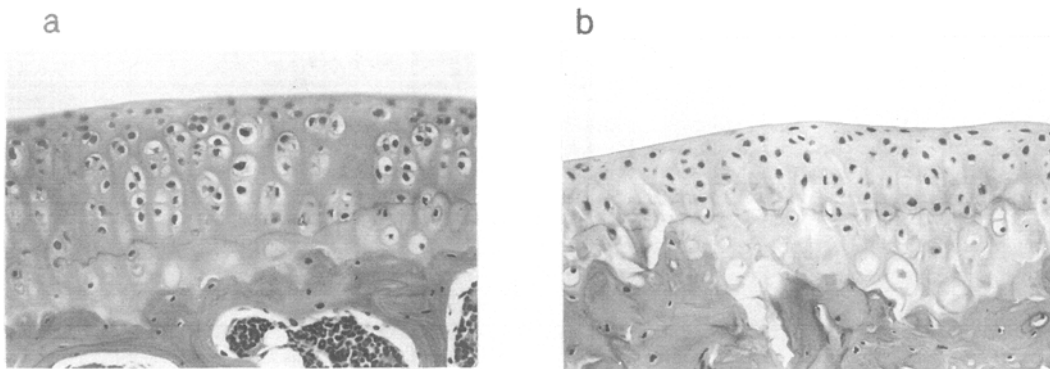


Fig. 4 Light microscopic findings of femur cartilage at the knee joint ($\times 100$).

The control subject at 8 months of age (a). The treated subject was fed 1% mixed sodium condensed phosphate (0.2% phosphorus) added pellet for 7 months. The figures disclosed a decrease in or irregular loss of the number of cells in the superficial, intermediate and radial strata.

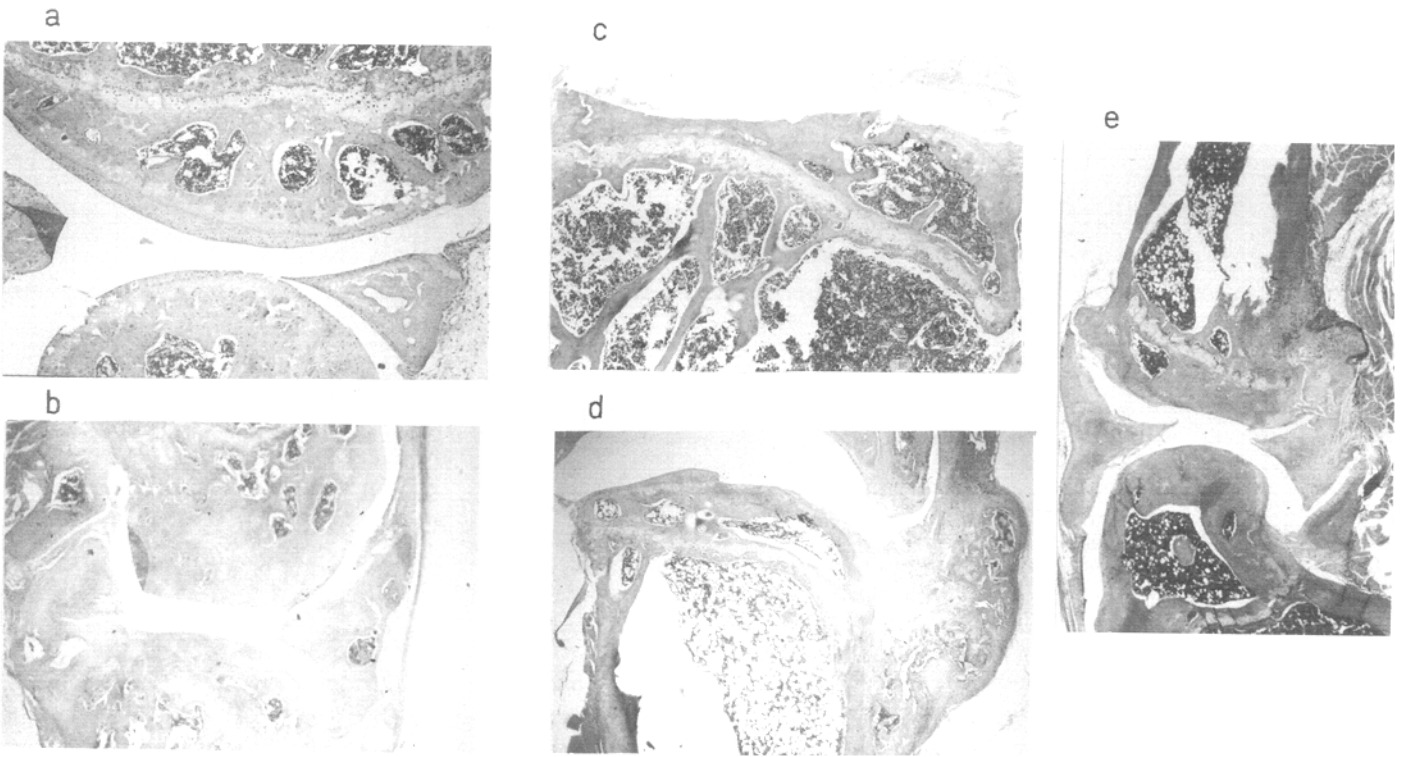


Fig. 5 Light microscopic findings of the knee joint of mice ($\times 20$).

Control subjects at 15 months of age (a). Treated subjects were fed 0.5% mixed sodium condensed phosphates (0.1%-P-feed) (b), 1% mixed sodium condensed phosphates (0.2%-P-feed) (c) and 2% mixed condensed phosphates (0.4%-P-feed) (d) for 14 months, and 2% mixed sodium condensed phosphates (0.4%-P-feed) for 22 months (e). The figures disclosed a defect in the subchondral bone, thinning of the cortex, reduction of the marrow trabeculas, ossification of the articular cartilage, disarticulation of the femur and the tibia at the knee and allopatic development of osteophytes.

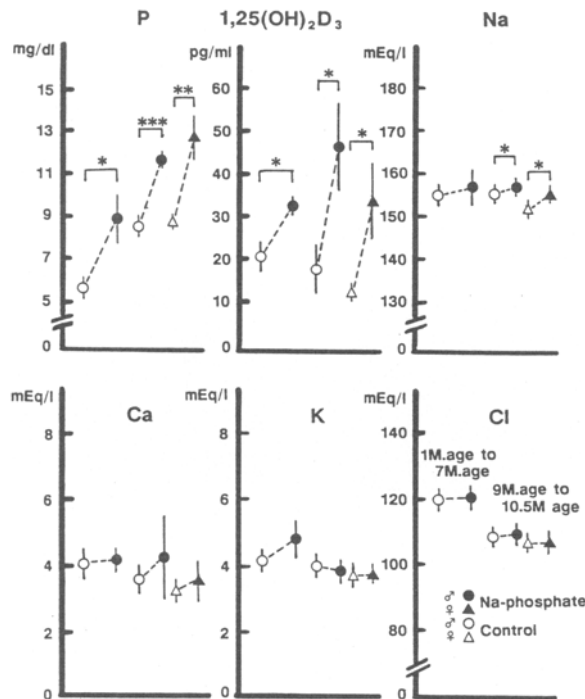


Fig. 6 Changes of P, $1\alpha, 25$ (OH)₂D₃, Na, Ca, K and Cl values in plasma of the mice treated with 1% mixed sodium condensed phosphates (0.2% phosphorus) for 5 months (from 29 days old to 6 months old) and 1.5 month (from 9 months old to 10.5 month old).

(\circ , \triangle : control group \bullet , \blacktriangle : condensed phosphate group)

Significant level $p < .01$ **, $p < .05$ * by t-test

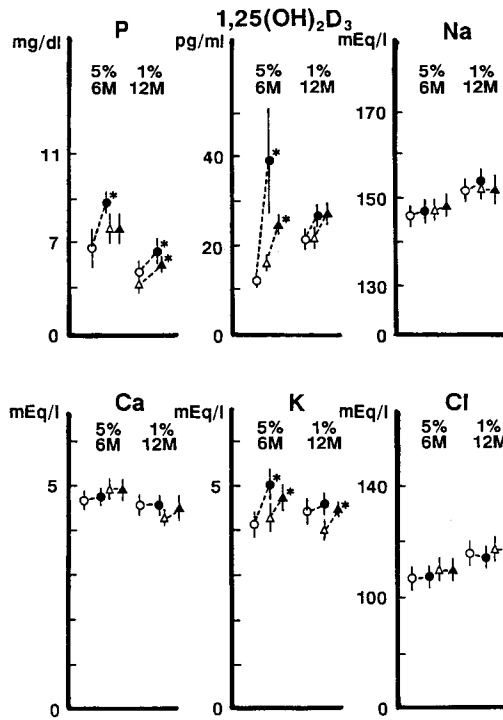


Fig. 7 Changes of P, $1\alpha, 25$ (OH) $_2$ D $_3$, Ca, K, Na and Cl values in plasma of the mice which were fed with 5% mixed potassium condensed phosphate added pellets (1% phosphorus) for 6 months, 1% mixed potassium condensed phosphate added pellets (0.2% phosphorus) for 12 months.

(○, △ : control group ●, ▲ : condensed phosphate group)

Significant level $p < .01$ **, $p < .05$ * by t-test

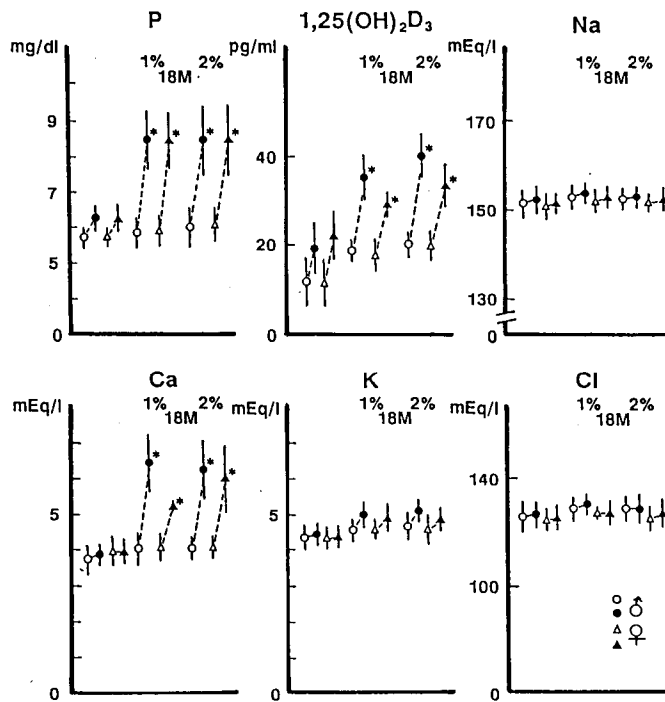


Fig. 8 Changes of P, $1\alpha, 25$ (OH) $_2$ D $_3$, Na, Ca, K and Cl values in plasma of the mice which were fed mixed calcium condensed phosphate 1% or 2% (0.2% or 0.4% phosphorus) added pellets for 18 months (from 29 days old to 19 months old).

(○, △ : control group ●, ▲ : condensed phosphate group)

Significant level $p < .01$ **, $p < .05$ * by t-test

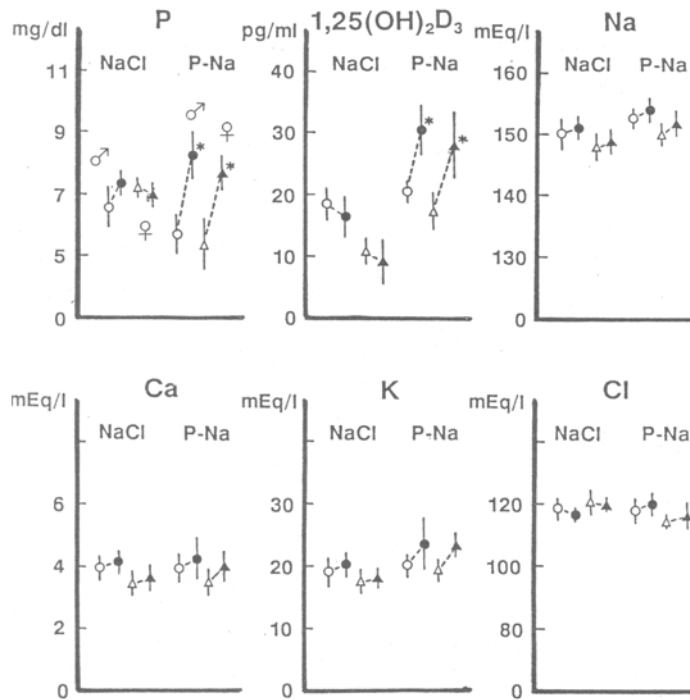


Fig. 9 Influences of 5% NaCl, 5% mixed sodium condensed phosphate (1% phosphorus) added pellets which were fed to the mice on P, Na, K, Cl, Ca and 1 α , 25 (OH)₂D₃ values in mice plasma.

(○, △ : control group ●, ▲ : condensed phosphate group)

Significant level $p < .01$ **, $p < .05$ * by t-test

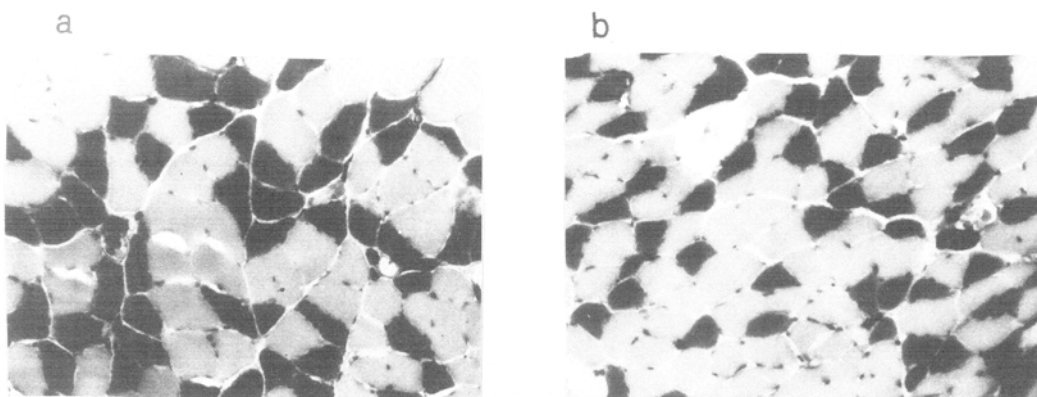


Fig. 10 ATPase-stained soleus muscle of mice.

Control subject at 15 months of age (a).

The treated subject was fed 2% mixed sodium condensed phosphate (0.4% P-feed) for 14 months (b).

Type I (light) and Type II (dark) fiber ($\times 70$).

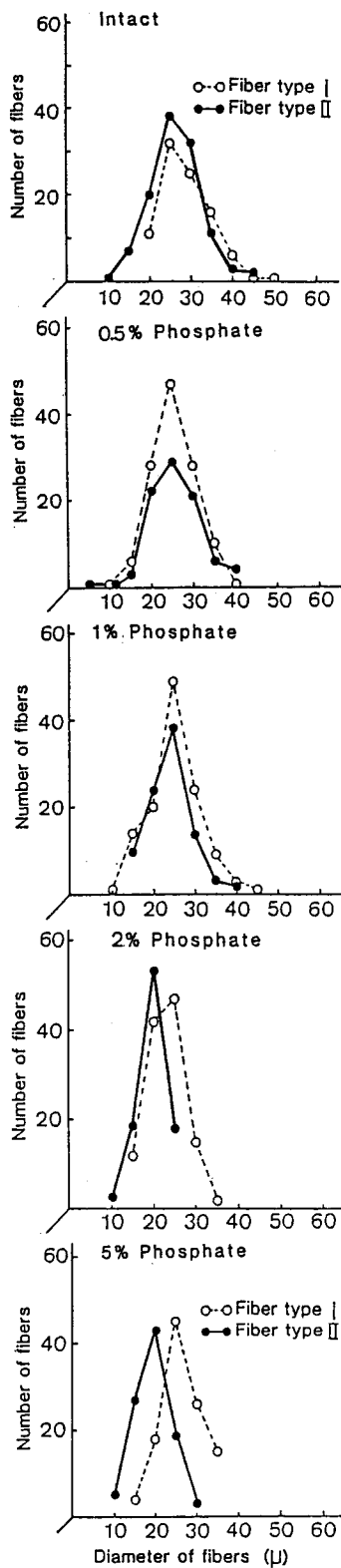


Fig. 11 The distribution of diameters of the soleus muscle fibers of mice.

Intact: Control group, mice of this group were fed pellets with no added phosphates for 425 days.
 0.5%, 1%, 2%, 5% phosphate: Each experimental group of mice were fed 0.5%, 1%, 2%, 5% mixed sodium condensed phosphate added pellets (0.1%, 0.2%, 0.4%, 1% phosphorus) for 425 days.

groups. These changes were more evident in the low P • long term administration group than in the high P • semilong-term administration group. Ca showed significant elevation, too, but the high P • semilong-term administration group did not show very clear changes. (Fig.8)

5) Pellets added with 5% mixed condensed Na-phosphates (P concentration was approximately 1%) and pellets mixed with 5% NaCl were given to the CF # 1 strain mice, male and female, 29 days old, and the administration was made for 6 months. Each phosphate administered group showed a significant elevation of P and $1\alpha, 25(\text{OH})_2\text{D}_3$, but the NaCl group did not show changes. (Fig.9)

5. Muscle examination

CE-7 pellets added with mixed condensed Na-phosphates at 0.5%, 1%, 2%, and 5% (P contents were approximately 0.1%, 0.2%, 0.4%, and 1%) were given for 400~450 days, and the subjects were sacrificed during 400 to 450 days in the order of a decreasing value of P concentration of the feed. Then diameters of 100 samples each from type I and type II fibers of the soleus of the hind leg were measured with a microscope. Regarding both type I and II fibers, the distribution curve of their diameters showed a peak and it moved to a shorter range (left) in accordance with the concentration of P added to the feed; particularly this change was evident with type II. Shrinkage of the diameters of the muscular fibers of the subjects treated with K-or Ca-phosphates was less than that treated with Na-phosphates.¹⁵⁾ (Fig.10, 11)

6. Histological findings

1) Soleus fixed by 10% formalin was embedded in paraffin in the usual method and a slice was examined. The shrinkage of the diameters of muscular fibers was observed and irregularity of the size and the internal nucleus of the fibers was observed. In the case of K-phosphates and Ca-phosphates, these changes were less than in the case of Na-phosphates.

2) Regarding kidneys, examinations were made on the mice which were administered with pellets added with 2% and 0.5% Na-phosphates (P contents were approximately 0.4% and 0.1%) for 13 months. In the subjects treated with either concentration of P, swelling, atrophy, and desquamation of glomerular capillaries, interstitial tissue inflammation, and development of cylindruria were noticed. In the case of the 22-month treatment, atrophy, desquamation, and incrustate of the basement membrane of glomerular capillaries, swelling of tubular epithelial cells, their thinning and desquamation, and development of cylindruria were observed; incrustate of the basement membrane was slightly greater. In a small number of the subjects, nephrosclerosis, and Ca deposition in uriniferous tubules were noted. Observations on the subjects fed with 5% phosphate added pellets (P content was approximately 1%) for 6 months showed that incrustate of bowman's capsule, swelling of tubular epithelial cells, and proliferation of mesenchymal cells differentiated into interstitial tissues were great. Some of the 12-month administered mice showed induration of glomerular capillaries and Ca deposition in uriniferous tubule. In the case of K-and Ca-phosphates, changes in the kidneys were weaker than the case of Na-phosphates. (Fig. 12)

3) Observations of the liver were made on the same subjects as those used for kidney observations. In the Na-phosphate, 2%

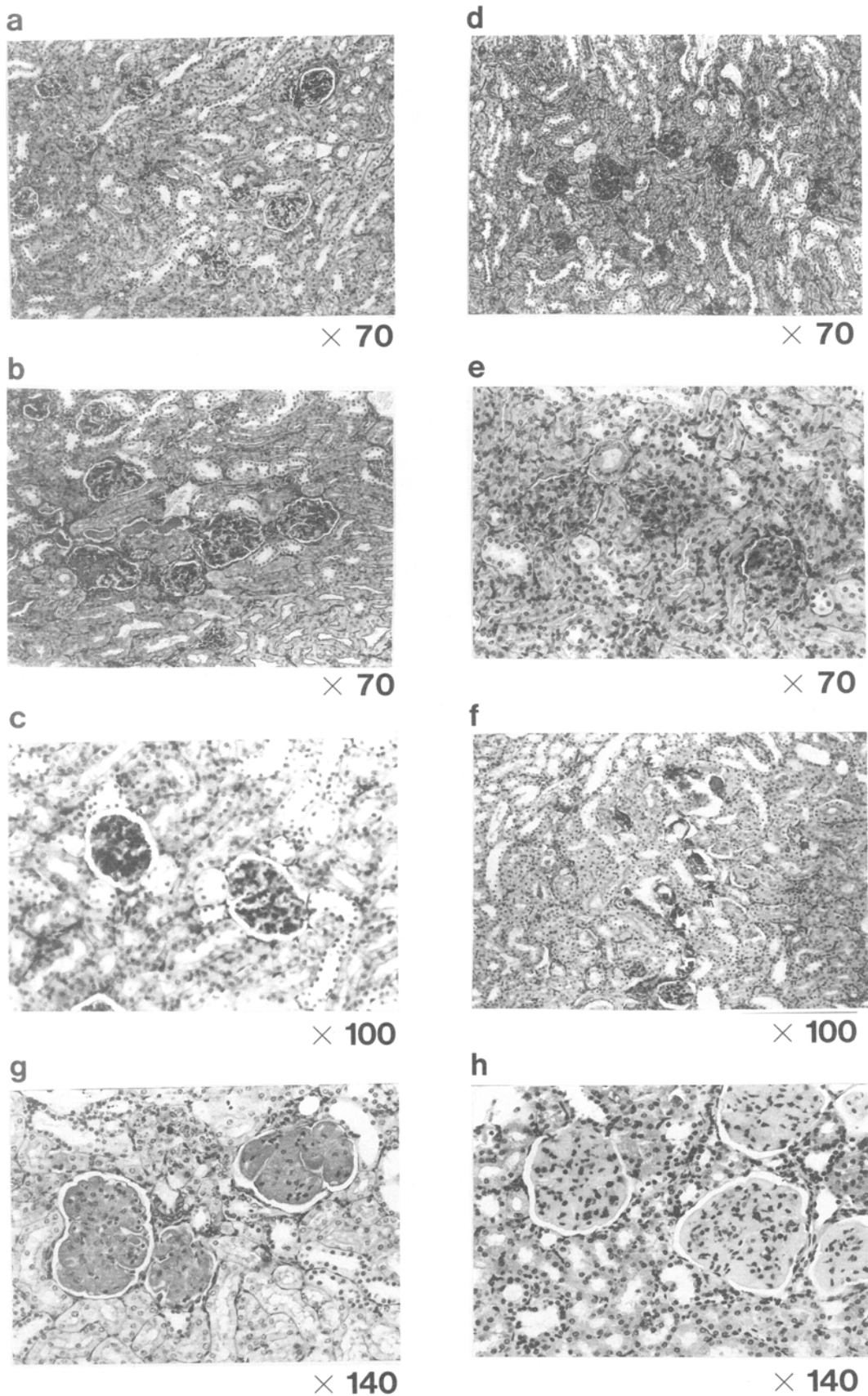


Fig. 12 Light microscopic findings of kidneys (PAS-stained).

Control subject at 15 months of age (a) and the treated subject was fed 0.5% mixed sodium condensed phosphates (0.1%-P-feed) for 14 months (b~f), and 22 months (g, h).

The figure disclosed cyclinuria (b), proliferation of mesangial cells (c), swelling of the glomerulus and induration of glomerular capillaries (d), incrassate and adherent of Bowman's capsule (e), calcium deposition in uriniferous tubules (f), and induration of glomerular capillaries (g, h).

and 0.5% (P content, 0.4% and 0.1%) 13-month groups, binucleate cell, nuclear size alteration, pyknosis, necrosis, interstitial tissue inflammation, activation of Kupffer cell mobilization, etc. were seen in both the 2% and 0.5% groups, but in the 0.5% group the findings were less obvious and in the 2% group they were at the medium degree. In the case of 22-month administration, karyolysis, pyknosis, necrosis, interstitial tissue inflammation, activation of Kupffer cell mobilization, hemosiderin deposition, etc. were seen at the medium degree in the 0.5% group and strongly in the 2% group. These findings were less obvious in K- and Ca-phosphates than in Na-phosphates.

Discussion

A food additive (referred to as FA hereafter) is a synthetic chemical substance to be added to foods for the purpose of production, preservation, and elevation of consumers' preference for the food. It brings economic effects and is a useful material for food hygiene. Presently, however, it is sometimes used for unnecessary purposes or in more than necessary amounts. Diversified and abundant information and advertisement, together with different ways of selling, have allowed a tremendous increase in the kinds and quantity of processed foods and have then induced an increase in the amount of consumption for convenience' sake. These circumstances have brought about a large amount of production of processed foods in the long run, and have presently resulted in a tremendous excess of supply which is termed 'well-fed and well-cladera'. Due to diversified chemical properties, condensed phosphates have a broad application field as FA and actually, they are used widely and abundantly. According to a survey by the Ministry of Health and Welfare of Japan,¹⁹⁾ limiting their usage to that of a binding agent, condensed phosphates have shown a great yearly increase over the past ten years. Another survey of processed foods was made group by group to find the inclusion of condensed phosphates and to measure the intake per day by school children and by general adults independently in Japan.²⁰⁾ The data disclosed condensed phosphate intake by children was much larger than by adults.²⁰⁾ There was a large amount of condensed phosphates used as a binding agent in the foods for which school children have a preference and the children's intake of condensed phosphates per day was greater, too. Also, other than FA, P is contained in the food itself and such P is much larger in amount than the Ca content.

The ratio of P to Ca content is 3~40 : 1 in many foods. There are reports from old times which say that the P intake from Japanese foods is between approximately 1300 mg and 1400 mg.^{21), 22)} As the present mean intake of Ca by Japanese is approximately 550 mg, assuming that P intake is 1400 mg, the ratio of P to Ca becomes about 2.5 : 1. It has been pointed out that, if this figure for P, here 2.5, becomes larger than 2, absorption and excretion of Ca become unbalanced and a functional aberration of the parathyroid is induced.²¹⁾ In other advanced countries, necessary P and Ca intakes are found to be equal.

Tripolyphosphate, a condensed phosphate in processed foods, mostly changes to dipolyphosphate when foods are processed. However, in the condition of low temperature or high temperature it has become apparent that the activity of enzymes to metabolize the phosphate lowers²³⁾ and it is highly

probable that tripolyphosphate remains in refrigerated or frozen or high-temperature treated foods. There are other condensed phosphates such as metaphosphates and pyrophosphate, and, further, diphosphate produced by the decomposition of tripolyphosphate is also condensed phosphate, and they have chemical properties as condensed phosphate. Consequently, as we observed in our study, we can interpret there is activity towards the living body inherently in processed foods.

To the maximum non-effect level (MNL), that is, the maximum quantity which has no effect even through a long term administration covering nearly the whole lifetime of small animals, the safety factor was applied taking into account the difference in species, and the acceptable daily intake (ADI) was determined. This value for all phosphates among FA including condensed phosphates has been decided at 70 mg/kg b.w./day in terms of total P. However, we do not know the indices employed or the size of the extent where they were chosen for observations to decide MNL which is the basis of ADI. We do not know, either, how great the estimated factor of safety was, nor do we know how absorbed and excreted amounts of the phosphate reported in the papers mentioned above were reflected in food safety or how they were determined to be a guide. Regarding ADI (70 mg P/kg b.w./day), as condensed phosphates have no standard usage as FA, supposing the safety factor was decided to be 100 times as big, that is, the maximum non-effect level is one hundredth, the MNL for small animals will be 7g P/kg b.w./day. The authors' MNL for small animals obtained through observations is less than 87 mg P/kg b.w./day. Since P is an essential element to the living body, it has been considered, so far, to be safe and its toxicity has not been widely discussed.

About 80% of the amount of P in the living body is contained in the bones and forms a complex with Ca (P : Ca = 1 : 2). P naturally associates with the movement of Ca which centers around the bones, and, hence, it is considered that observations of the bones are essential for observing the effects of condensed phosphates on the living body. High P intake causes an elevation of the P level of blood and this stimulates acceleration of the parathyroid function.²⁴⁾ Then this brings about an elevation of the parathyroid hormone level of serum¹⁷⁾ and the hormone induces emigration of bone Ca. Also, the hormone activates renal 1α -hydroxylase and, hence, $1\alpha, 25$ (OH)₂D₃ increases. $1\alpha, 25$ (OH)₂D₃ accelerates production and absorption of Ca combined protein in the small intestine and further induces acceleration of reabsorption of Ca and depression of reabsorption of P at the uriniferous tubules. When this chain of processes becomes chronic, a load is placed on the renal function and glomerular capillaries and uriniferous tubules are overloaded. As a result, it is conceivable that changes in renal tissues take place. Parathyroid perceives a slight decrease in blood Ca and induces functional acceleration and secretes PTH. When condensed Ca-phosphate was administered in the present investigation, irrespective of a rise in Ca level of blood, the $1\alpha, 25$ (OH)₂D₃ level rose. Thus, in the case where condensed Ca-phosphate was administered the P level of blood became apparently high just like in the case of condensed Na-phosphate and K-phosphate. Consequently, it is considered that a rise in the parathyroidal function for Ca homeostasis is stimulated not only by a decrease in the Ca level of blood but also by an increase in the P level of blood and, in addition, an im-

balance between the P and Ca levels plays the role of a trigger.

There is a strong ability of condensed phosphates to form chelate facilitated excretion of Fe into urine and feces,¹⁴⁾ and, according to papers by other researchers, accelerated excretion of Zn.^{25), 26)} Moreover, it affects the movement of minerals in the living body,²⁷⁾ and leads to a deficiency of essential trace metals in the living body. It is considered that the occurrence

of bone in abnormal locations, the formation of osteophytes, and Ca deposition in the achilles tendon and in the kidneys which were seen in the present studies are the results of the effects of condensed phosphates on the distribution of Ca. In view of these results, it would be necessary to reconsider the extent and the amount of FA usage, as well as MNL, which is the basis of determining the ADI of condensed phosphates.

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