Effect of Alkali on Metastatic Calcification Produced by Hypervitaminosis D

Pages with reference to book, From 345 To 348

Gulshan Naheed (Present Address: Khyber Medical College, Peshawar.)
N.A. Jafarey (Dept. of Pathology, Basic Medical Sciences Instiute, Jinnah Post graduate Medical Centre, Karachi.)

Abstract

The effect of alkali (Sodium Bicarbonate) on metastatic calcification produced by Hypervitammosis D (ergocalciferol) was studied in rats. Six organs e.g. kidneys, heart, lungs, stomach, aorta and liver of the rats were examined for evidence of calcification.
Six out of ten rats receiving vitamin D alone and seven out of ten rats receiving vitamin D and sodium-bicarbonate in combination, showed calcification of various organs. There was no significant difference in the amount and distribution of calcification between the two groups, except in the lungs where calcification was more severe in the animals receiving alkali along with vitamin D (JPMA 34 345, (1984).

Introduction

Vitamin preparations are liberally prescribed by practicing doctors and used by people enthusiastically in the belief that these improve health in disease or otherwise. Most of the people indulge in unauthorized self medication as these vitamin preparations are available without medical prescription and are called tonics. What is not realised however is that there is a potential danger of serious toxicity if some of these are taken in excessive quantities or for a longer period of time. Vitamin D when taken in large amounts is known to be toxic[1]. The most common toxic effect is metastatic calcification of soft tissues of the body and occurs in individuals of any age. In addition metastatic calcification has been obs'rved in patients with chronic peptic ulcer who have used milk and alkali for prolonged periods.[2] This is known as milk alkali syndrome. Anderson[3] has described the association of pulmonary alveolar microlithiasis with milk alkali syndrome.
Genetatly calcification is seen in organs where relative alkalinity is produced such as renal tubules, pulmonary alveolar walls and gastric mucosa. These observations suggest that administration of alkali probably contributes towards calcification. It was therefore decided to study the effect of alkali on metastatic calcification produced by excessive doses of vitamin D.

Material and Methods

Thirty five adult female rats (Table I) were used in the experiment. Animals were divided into four groups. Group A served as control and had five animals. Groups B, C and D were experimental and each included ten animals. They weTe given the treatment as per schedule shown in table I.

Table –I Experimental Groups.

Group	Number of Animals	Treatment given per Animal per day for 8 weeks
A	5	Control
B	10	10,000 Units of Vitamin D
C	10	10,000 Units of Vitamins D+ 150 mg sodium bicarbonate
D	10	150 mg sodium bicarbonate
Total	35	

Drugs used were vitamin D_2 and sodium bicarbonate. Vitamin D_2 or ergocalciferol was water soluble and the biological assay was 1.15400 units per gram or 1000 units per mg. 10,000 units of vitamin D was given per animal per day to groups B and C and 150 mg of sodium bicarbonate per day to each animal in groups C and D. These drugs were administered for a period of 8 weeks.

At the end of the experimental period, the animals were sacrificed, and their hearts, lungs, aorta, kidneys. stomachs and liver were presevedin 10% buttered tormaline. At autopsy the weights and the naked eye appearances of the whole organ and cut surfaces of liver, heart, kidneys, stomach and lungs of each animal were recorded. Suitable blocks of these organs were taken after processing the tissues in different grades of alcohol. Seven micron thick sectionscut and were stained with haematoxyleneeosin and other special stains for microscopic examination. The special stain used was von- kossa's stain for demonstration of calcium. Go mon's Reticulum and trichrorne 6tans were also used fcr the demonstration of structural architec ture and fibrosis.

All the sndes were examined with the help of a light microscope for evidence of calcification in different organs. Blood was also taken from each animal at the start and again at the end of the e perimental period to determine the serum ealeium levels. This was done by rapid coloil metric method adopted by Gindler and King in 1972 and was available in the foim of kit prepared by bioMerieux Company.

Results

There was marked reduction in the body weights of animals that received vitamin D alone (Group B) and in combination with alkali (Gronp C). The serum calcium levels on the other hand increased markedly (Table II).

Table II

Mean Serum Calcium Levels of the Control and Experimental Groups.

Group	Number of Animals	Treatment given	Mean serum calcium level mg% ± S.D. at start of experiment	at end of experiment	P Value
A	5 (1–5)	Control	9.55 ± 0.93	9.8 ± 0.8	N.S.
B	10 (11–20)	Vitamin D	7.96 ± 1.5	12.7 ± 0.87	<0.001
C	10 (21–30)	Vitamin D + Alkali	7.32 ± 1.23	11.9 ± 1.08	<0.001
D	10 (31–40)	Alkali	9.28 ± 0.82	8.8 ± 0.52	N.S.

Key : N.S. = Not significant. S.D. = standard deviation < = less than

At the start of the experiment the mean serum calcium level of group B was 7.96 ± 1.5 mg percent and group C was 7.32 ± 1.23 mg percent. At the end of the experimental period the mean serum calcium level of group B was 12.7 ± 0.87 mg percent and group C was 11.9 ± 1.08 mg percent.

The difference between the serum calcium levels at the start and the serum calcium levels at the end of experiment was statistically significant in animals given vit D (group B P <0.001 and group C P <0.001). No significant difference in the calcium levels were seen in control animals and those given alkali alone (group D).

Microscopic Finding

Animals of groups B & C showed calcification of various organs. Six animals out of ten in group B receiving vitamin D alone and seven animals out of ten in group C receiving vitamin D and alkali together , showed calcification of heart, Kidneys, lungs, aorta and stomach in different combinations. None of the animals showed calcification in the liver. The control animals of animals given alkali alone showed no calcification at all.

Kidneys

In the kidneys calcification was seen in the distal convoluted tubules and collecting ducts mostly (Fig. 1).

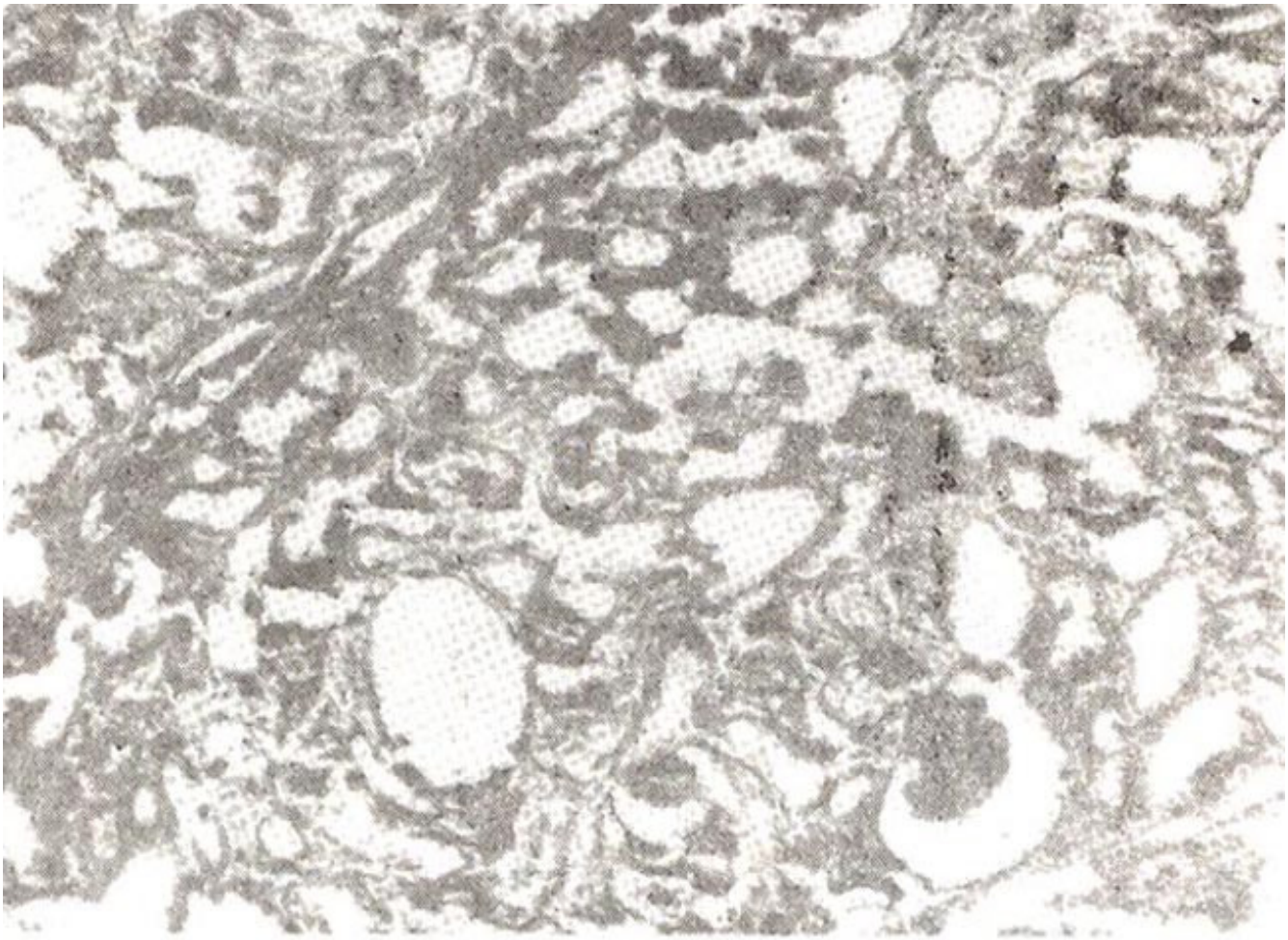
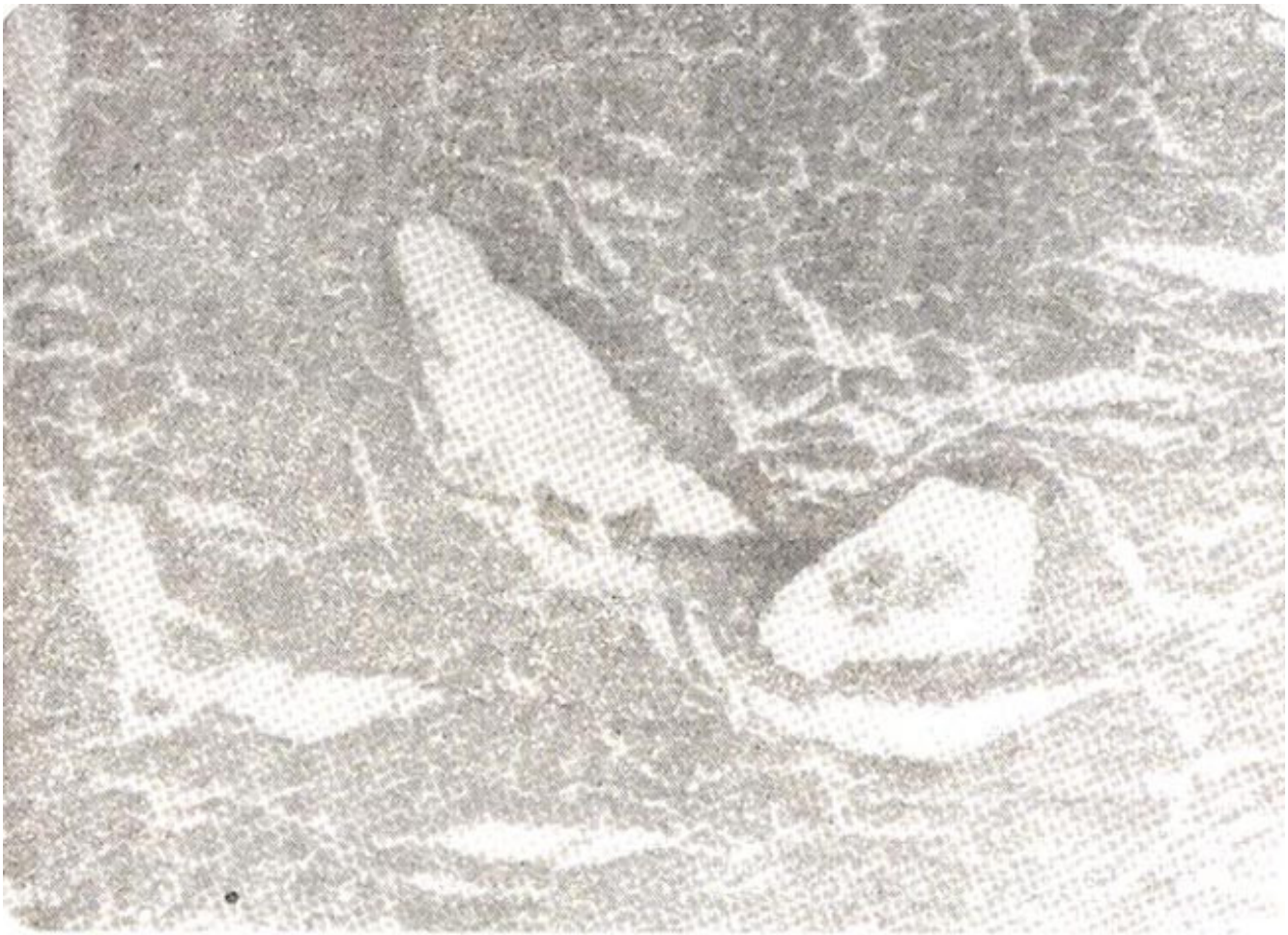


Fig. 1. Photomicrograph showing calcification in the collecting ducts of Kidney. Van Kossas Stain (X 106) .

Loops of Henle and proximal convoluted tubules showed deposits of calcium salts to a lesser degree. Von- kossa's stain indicated calcium salts as black deposits (Fig 1) and Haematoxylene and eosin stain showed calcium as purple. Five out of ten animals (50 %) in group B and four (40 %) in group C, showed calcification of the kidney parenchyma in different patterns.

Heart

Purple deposits of calcium salts were seen in the myocardium, small coronary blood vessels and ascending aorta (Fig. 2).



Fig, 2. Photomicrograph showing calcification of coronary arteries H&E. (X 106).

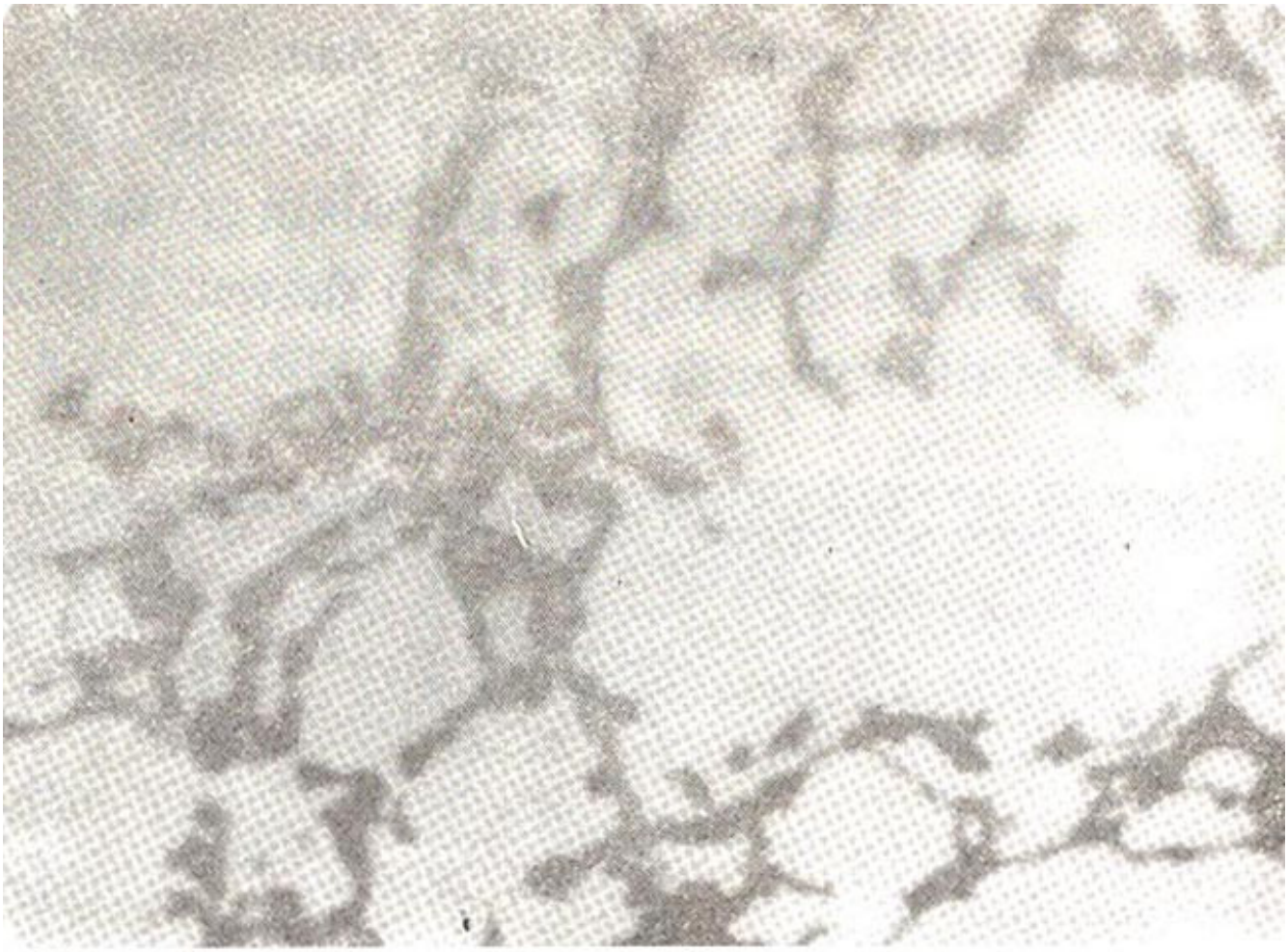
In areas where calcium salts were deposited muscle fibers showed atrophy and degeneration. A few giant cells, lymphocytic infiltration and increased connective tissue were also seen. Trichrome stain showed increased connective tissue in and around the calcified area. Four out of ten animals in group, B (40%) and three in group C (30%) showed calcification of the myocardium.

Aorta

The aorta showed deposits of calcium salts in the tunica media which were seen as blue (on H and E) and black deposits after von Kossa's stain. In some cases the intima showed focal areas of rupture. Six animals in group B (60%) and seven animals in group C (70%) showed calcification of the aorta.

Lungs

Sections of lungs of all the animals including the control group showed mild to heavy pen-bronchial lymphocytic infiltration. Two of the animals in group B (20%) and five animals in group C (50%) showed heavy deposits of calcium along alveolar walls and septa in the form of linear streaks and amorphous deposits (Fig.3).



Fig, 3. Photomicrograph showing calcification in the alveolar walls and septa, Von Kossa's stain (X 106).

Stomach

In the stomach deposits of calcium salts were seen in the muscle coat, blood vessels of submucosa and basement membrane of mucosal glands. Four animals in group B (40%) and two in group C (20%) showed calcification in the stomach. Liver Calcification of the liver was not seen in any animal.

Discussion

The dose of vitamin D and the duration of experiment was enough to produce heavy calcification by itself. So it is difficult to evaluate if the addition of alkali had any potentiating effect or not.

The present finding is different from another study where the soft tissues of dogs receiving both vitamin D and alkaline salts revealed more calcification than those receiving only vitamin D[4]. In our study this was true only for the lungs. Enhancement of soft tissue calcification by oral in-take of alkali and intravenous injection of sodium-bicarbonate has been served, after which excess of calcium and phosphorus was found in the lungs and kidneys.[4]

In this study, there was variation in the development of calcification and the degree of calcification which could be due to one of the following reasons.

There is always great variation between individual animals and species as a whole as regards susceptibility and sensitivity to the toxic action of vitamin D[5]. Toxicity with 50,000 units and 100,000 units of vitamin D respectively has been reported.

Se adly the presence of regulators of calcification such as pyrophosphates, diphosphonate and polyphosphates in the blood and tissues modify the response to the action of vitamin D. The minimum value of the product of calcium and phosphorus was raised from 53 to 105 mg/l00 ml when a solution containing pyrophosphate was added to the mixture of calcium and phosphate.[6]

Patients with chronic peptic ulcer taking alkali and antacids frequently for the relief of pain should check their renal function tests off and on for any microscopic haematuria or unsuspected renal function impairment.. Also the alkali treatment of food stuff should be avoided as alkali treatment of proteins forms lysino alanine, (LAL) which is responsible for renal cytomegalia[7].

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