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Influence of Acid and Phosphate on Metastatic Calcification.

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Metastatic calcification in otherwise apparently healthy soft tissue has been occasionally observed accompanying destructive bone lesions in cases in which there is associated renal disease. The factors responsible for this deposit have not been entirely evaluated. Rabl[1] fed mice alternately acid and alkaline diets rich in calcium and phosphorus and observed typical metastatic calcification in kidneys, heart, stomach, lungs and arteries. He explained the deposition of calcium on the basis of assumed rapid alterations in the alkalinity of the blood and resulting variations in calcium solubility. Butler,[2] Dreyfuss,[3] and Kleinmann[4] confirmed Rabl's observations but found that alternation was not necessary and that acid diets rich in calcium and phosphorus, when taken alone, were also followed by metastatic calcification. In their experiments it is not clear to what extent the deposit is due to the acidity as such and how much must be attributed to the fact that acidity was produced by phosphoric acid. The following observations were undertaken to determine whether high phosphate intake was necessary to the production of metastatic calcification according to the method used by Rabl.

The diets were modeled after those of Rabl. Each diet contained 100 parts of sugar and 100 parts of dried whole milk, containing 1.357% of calcium and 1.660% of phosphorus. Further adjustments were made as follows: (1) High phosphorus, high calcium acid diet—10 parts of calcium phosphate and 10 parts of 85% phosphoric acid were added. (2) High phosphorus, high calcium alkaline diet—10 parts of calcium phosphate, 200 parts of saturated solution of sodium acetate and 300 parts of cooked mashed potato were added. (3) High phosphorus, high calcium neutral diet—10 parts of calcium phosphate were added. (4) High calcium acid diet—10 parts of calcium lactate and 10 parts of ammonium chloride were added. (5) High calcium alkaline diet—10 parts of calcium lactate, 200 parts of saturated solution of sodium acetate, 300

[1] Rabl, C. R. H., *Klin. Wochenschr.*, 1923, **2**, 202.

[2] Butler, M., *Proc. N. Y. Path. Soc.*, 1924, **24**, 79.

[3] Dreyfuss, W., *Beit. z. path. Anat. u. allg. Path.*, 1927, **76**, 254.

[4] Kleinmann, H., *Biochem. Z.*, 1928, **196**, 161.

parts of cooked mashed potato were added. (6) High phosphorus acid diet—5 parts of disodium phosphate and 10 parts of 85% of phosphoric acid were added.

Young adult white rats were fed one or another or a combination of these diets for a period of 15 days, after which they were killed. The following organs were studied microscopically: lung, liver, kidney, heart, pyloric portion of stomach, spleen, pancreas, thoracic aorta, skeletal muscle, skin, and in a few instances testicle, lymph node, brain and adrenal. Sections were fixed in 4% neutral formalin; paraffin sections were stained with silver nitrate and hematoxylin and eosin, and with silver nitrate and alum carmine.

Forty young adult white rats were used and all survived until the end of the experimental period. The results of feeding the various diets and combinations of diets are shown in Table I. Soft tissue

TABLE I.
Soft Tissue Calcification in Rats.

Diet	No. Animal	Kidney	Heart	Stomach	Artery and Location	Other Organs
High Calcium	1	+++	0	0	0	0
High Phosphorus	2	++++	+	++	++ Pulmonary	0
Acid	3	++	+	+	++ Renal	0
4 Rats	4	++	0	0	+ Pulmonary	0
					+ Stomach	0
					0	0
High Calcium	1	+	0	0	+ Pulmonary	0
High Phosphorus	2	+++	0	0	+ Pulmonary	0
Alternating acid	3	+	0	0	++ Pulmonary	0
and alkaline diets every 2 days 4 Rats	4	+	0	0	0	0
High Calcium High Phosphorus Alkaline—5 Rats	all	0	0	0	0	0
High Calcium High Phosphorus Neutral—5 Rats	all	0	0	0	0	0
High Calcium Acid—12 Rats	all	0*	0	0	0	0
High Calcium Alternating acid and alkaline diets every 2 days—6 Rats	all	0	0	0	0	0
High Phosphorus Acid—6 Rats	all	0	0	0	0	0
Normal Rats	all	0	0	0	0	0

* In one kidney section there were 3 tiny questionable calcium concretions in tubules. Duration of each experiment—15 days.

calcification was observed only with the high calcium, high phosphorus acid and the high calcium, high phosphorus alternating diets, and was more marked and widespread with the former. No deposit was found when phosphate was not present in the diet. Of the individual organs, the kidney and pulmonary artery were most frequently involved; the most extensive calcification was seen in the kidneys.

The calcium was deposited as gross concretions, easily seen under low power of the microscope as dense black masses in the stained sections; the calcium deposits were all apparently extracellular. In all organs, the tissue surrounding the calcium concretions was the site of a cellular reaction consisting chiefly of infiltration of lymphocytes. In the heart muscle, in addition to the lymphocytic reaction, there was necrosis and early scarring in the immediate vicinity of the calcium deposit. In the kidneys, the most extensive deposition of calcium was noted in the region of the junction of medulla and cortex and extended into both of the latter; calcium was deposited for the most part in tubules; to a lesser extent in the interstitial tissue. No deposits were seen in the glomeruli. In the stomach sections, calcification was noted in the muscular coat and in one case in the wall of the small artery. In the pulmonary, renal and gastric arteries which showed calcification, the deposits were located beneath the intact intima, in the muscular layer. No deposits were seen in any of the sections of the aorta. Several of the lung sections showed calcium deposits in cartilages of the larger bronchi; this was also seen in several of the control rats and, therefore, was not considered significant.

The calcium deposits could be removed from sections by treatment with 5% hydrochloric acid before staining with silver nitrate. In unstained sections containing large amounts of calcium, no gross or microscopic bubbling could be detected upon treatment with 5% hydrochloric acid. This was interpreted as indicating that significant amounts of carbonate were not present and that the calcium was deposited chiefly as the phosphate salt. Iron stains were done on a number of sections; in all organs showing calcium with the silver nitrate stains, small amounts of iron were demonstrated in the areas of calcium deposition.

As controls, the organs of 6 healthy young adult white rats were sectioned and stained; in none of them was any calcification observed except in bronchial cartilages, as noted above.

Serum calcium and serum phosphorus values were determined on blood samples obtained at the time the rats were sacrificed. There

was no gross deviation from the normal in the animals in which soft tissue calcification was observed.

The probable importance of phosphate in the deposit of calcium in soft tissues has been shown by Smith and Elvove,[5] who found in their experiments with viosterol poisoning that the relationship of calcification in tissue to phosphate values in serum could be clearly established. High serum phosphate accompanied by only a slight increase in serum calcium generally resulted in great calcification of soft tissues. On the other hand no abnormal deposition of calcium was found in any case in which the serum phosphate values were low. Shelling[6] found that animals fed with a minimum of calcium and a high phosphorus ration were extremely susceptible to viosterol and exhibited most profound calcification of soft tissues. He showed further that increased phosphorus retention characterized the metastatic calcification observed with hypocalcemia in parathyroidectomized dogs. Phosphate retention is a prominent feature in the terminal stages of nephritis. Following overdosage with parathormone or with irradiated ergosterol there is in the terminal stages an increase in serum phosphate. Since calcium metastases are recognizable only at autopsy, it seems possible that in these conditions the deposit occurs late and only after phosphate retention has become an important factor.

The experiments here reported indicate that in the development of metastatic calcification an excess of calcium, of phosphorus and of acid is necessary, and that if any one of these is not present in excess soft tissue calcification does not occur.

It is suggested that conditions of these short-term experiments in rats may be fulfilled for the development of metastatic calcification in certain patients who have a rarefying bone disease and a renal lesion, the former acting as a source of excess calcium, the latter resulting in acidosis and phosphate retention.

Summary. 1. Rats fed high calcium, high phosphorus, acid or alternating acid and alkaline diets for a period of 15 days, show extensive soft tissue calcification, confirming previous experiments by others. 2. With acid or alternating diets high in calcium but not rich in phosphorus, no calcification takes place. 3. With an acid diet rich in phosphorus but without added calcium, calcification does not occur. 4. Neutral or alkaline diets rich in calcium and phosphorus do not result in calcium deposition. 5. An excess of calcium phosphorus and acid is necessary for the production of metastatic

[5] Smith, M. I., and Elvove, E., *Pub. Health Rep.*, 1929, **44**, 1245.

[6] Shelling, D. H., PROC. SOC. EXP. BIOL. AND MED., 1930, **28**, 298.

calcification under the conditions of these experiments. Neither acidity nor rapid alternation between acidity and alkalinity is sufficient to cause significant calcification unless both calcium and phosphorus are present in excess. 6. The presence of iron was demonstrated wherever calcium deposits occurred.

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Current Intensity Factor in Electrocution of Paramaecia.

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The purpose of the present work is to determine in *absolute values* the intensity of current required to pass through a Paramaecium to disrupt it.

The organism, *Paramaecium caudatum,* cultured for 5 to 6 days in hay infusion, which consisted of Timothy hay boiled for 5 minutes in 100 cc. of distilled water and diluted to 500 cc. was introduced into a glass tube of known diameter and length. The tube was placed horizontally on a disc of hard rubber, the ends being immersed in big drops of culture medium into which the electrodes carrying the current (D.C.) were also introduced. The disc, placed on the stage of the microscope, was hollowed in the middle so as to allow the light from the condensor to pass through. Rheostats and milli- or microammeters, in series, completed the circuit. When a paramaecium came in sight in the tube the circuit was closed and the data taken. Selecting only those experiments in which the organisms were disrupted instantaneously (in less than 1/5 of a second) a "Band of killing intensities", extending from the intensities that killed about 5% to those that killed about 95%, was established for a set of tubes varying widely in diameter.

The following results were obtained: (cross sectional area of the tubes less cross-sectional area of the organism, in mm^2, in line A; band of killing intensities in microamperes, in line B).

A	0.14	.047	.048	.057	.277	.410	.488	.579	.827	1.126
B	7-10	9-13	9-11	12-19	35-68	44-62	59-66	85-95	100-106	144-186

A	1.192	2.258	3.786	4.128	5.461	6.502
B	130-136	353-375	539-631	650-677	854-887	1042-1097

The average of the 2 extreme intensities of the "band", plotted on the curve, (Fig. 1) show an evident linear relation.