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INVESTIGATIONS ON THE SOLUBILITY OF CALCIUM PHYTATE

BY

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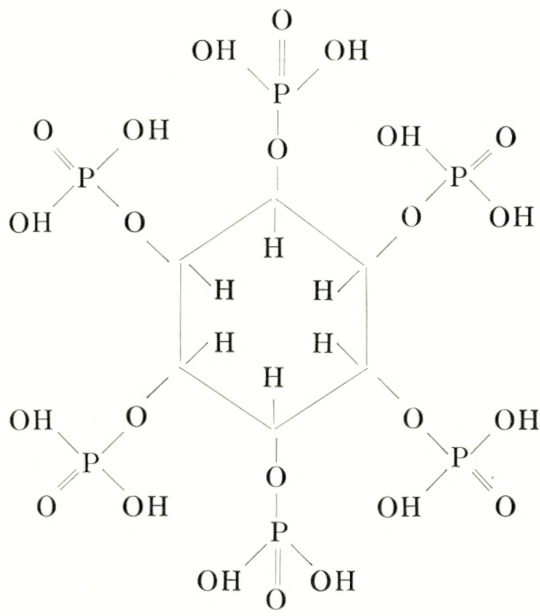
Phytic acid is the hexaphosphoric ester of inositol (Posternak (1921)): $C_6H_6(O \cdot PO(OH)_2)_6$,



and is thus a 12-basic acid; this acid is found particularly in cereals, 60 to 85 per cent. of the phosphorus here occurring as phytic acid (J. G. A. Pedersen (1940)). Phytic acid is not absorbed from the alimentary canal, and in the intestine of animals and man it is only to a slight extent split into inositol and phosphate (Starkenstein (1910)).

In 1920 Mellanby demonstrated that cereals contain varying amounts of an "anticalcifying factor" which favours the development of rickets. Bruce and Callow (1934) experimented with a diet which was rich in cereals, but poor in phosphate; their

results implied that the rachitogenic action of the cereals depends on their content of phytic acid, and that the rickets developed was due to lack of absorbable phosphate. In 1939 HARRISON and MELLANBY put forth a new theory regarding the effect of phytic acid. Their experiments support the assumption that the solubility of the calcium salt of phytic acid is so slight, that the absorption of calcium is impaired, because calcium phytate is precipitated in the intestine. Experiments on pigs by PEDERSEN (*l. c.*) have further supported both theories; he found that the former mode of action prevailed on a diet high in calcium, whilst the latter effect was the most pronounced on a diet low in calcium.

BRUCE and CALLOW's theory has been supported by various experiments, whilst MELLANBY's theory still rests on an assumption, as determinations of the solubility of calcium phytate have not so far been published.

It has been the aim of the present work first to determine the compositions of the calcium salts of phytic acid, when precipitated under conditions similar to those found in the intestine, and secondly to determine the solubility of the said salts.

1. Composition of the Calcium Salts of Phytic Acid.

The raw material used was sodium phytate, prepared from wheat bran according to the method of POSTERNAK (1921). The colourless crystalline sodium salt was recrystallised three times from water. For analysis it was dried at 120° C., by which 35 out of the 38 molecules of crystal water disappear.

	C	H	P	Na	H_2O (120° C.)
Found per cent.	7.48	1.22	19.14	28,2	38.8
Calculated for $C_6H_6O_{24}P_6Na_{12}, 3\ H_2O$ per cent....	7.36	1.23	19.02	28.2	39.2

Preparation of the Barium Salt of Phytic Acid.

A solution of sodium phytate, about 0.1 M, was brought to $p_H = 1$ with 1 N HCl. An equal volume of barium chloride solution, about 0.5 M, was added. After some time a white, crystalline precipitate began to form. The precipitation was

finished in about twelve hours. The salt was analysed after drying at 120° C.

	P	Ba
Found per cent.	17.62	38.3
Calculated for $C_6H_{12}O_{24}P_6Ba_3$ per cent.	17.45	38.6

If crystallization takes place at p_H above 2, the barium content is higher than corresponding to the above formula.

Preparation of Phytic Acid.

The barium salt was suspended in water and heated to 60° C. 0.5 M H_2SO_4 in a quantity determined by calculation, was added slowly during vigorous stirring. The barium sulphate formed was separated by filtration, and the clear filtrate was evaporated in vacuo until it was 1 M in phosphorus. This solution does not give the inorganic phosphate reaction with molybdate reagent. The phosphorus content was determined after destruction of the organic matter, and the solution then was diluted to be 0.6 M in phosporus, i. e. 0.1 M in phytic acid.

Preparation of the Calcium Salt of Phytic Acid.

30 ml. 0.1 M phytic acid were mixed with 100 ml. 1.0 M NaCl. The p_H of the solution was adjusted to 3.7 (glass electrode) by adding 0.5 M NaOH. Addition of 10 ml. 1.8 M $CaCl_2$ now caused a drop in p_H to about 2.7, without precipitation occurring. During vigorous mechanical stirring and aeration with CO_2-free air, 0.5 M NaOH was slowly added until the desired p_H was reached. Stirring and aeration was continued for one hour. The precipitate was filtered off by means of a Jena glass filter No. 11 G 3 and washed with three times 15 ml. boiling distilled water and dried at 105° C.

Analytical methods.

About 300 mg. of calcium phytate were ashed with sulphuric acid, nitric acid, and perchloric acid; the mixture was aerated during the ashing in order to prevent bumping. The precipitated calcium sulphate was dissolved by boiling with about 50 ml. 2 per cent. hydrochloric acid. The calcium content of this

solution was determined gravimetrically as CaC_2O_4, H_2O, and the phosphorus as NH_4MgPO_4, $6\,H_2O$, according to the methods of WASHBURN and SHEAR (1932). The accuracy of the methods was determined through eight analyses on solutions with known contents of calcium and phosphorus. The standard deviation of 8 calcium determinations was 0.25 per cent., and the standard deviation of 8 phosphorus determinations 0.30 per cent. The water content was determined by drying over P_2O_5 in vacuo at 200° C. (boiling benzyl alcohol). The compound was not discoloured.

Table 1 gives the results of the analyses of the calcium salt precipitated at varying p_H.

Table 1.

Precipitation of Calcium Phytate at Varying p_H.

Precipitation conditions	p_H	Ca p.c.	P p.c.	H_2O p.c.	P/Ca Mol
Precipitated at room temperature Stirred for one hour after precipitation	2.75	19.04	21.72	—	1.475
	3.01	19.25	21.30	—	1.430
	3.45	19.90	21.06	—	1.366
	3.70	20.12	21.37	4.66	1.370
	4.11	21.15	20.74	5.22	1.267
	4.25	21.76	20.60	5.38	1.223
	4.79	22.10	20.65	5.47	1.208
	5.30	21.86	20.62	5.82	1.216
	5.52	21.49	19.81	7.38	1.190
	6.03	21.79	20.11	5.67	1.192
	6.50	21.82	20.23	6.21	1.196
	6.92	21.95	20.21	5.81	1.188
	7.27	22.06	19.61	6.42	1.149
	7.85	22.45	18.72	6.90	1.077
	8.31	22.68	18.44	6.60	1.051
Precipitated at room temperature Stirred for 12 hours at 37° C.	3.72	20.06	21.27	—	1.370
	4.19	20.61	20.89	—	1.308
	4.85	21.21	20.05	—	1.220
	5.58	21.21	19.88	—	1.210
Precipitated at 100° C. Stirred for one hour at 100° C.	3.94	19.15	20.10	—	1.355
	4.12	21.24	20.72	—	1.260
	4.82	21.92	20.53	—	1.210
	5.47	22.10	20.37	—	1.190

Table 2 gives the results of some experiments in which the ratio P/Ca was varied in the solution. The table shows that the composition of the salt is independent of this ratio.

Table 2.
Precipitation of Pentacalcium Phytate, the Ratio P/Ca being Varied in Precipitation Solutions.

Precipitation conditions	P/Ca[1] Mol	p_H	Ca p.c.	P p.c.	P/Ca[2] Mol
Precipitated at	2/1	5.05	22.10	20.71	1 209
room temperature	1/2	5.05	22.00	20.64	1.211
Stirred for one hour	1/3	5.05	21.94	20.32	1.194
after precipitation	1/4	5.05	22.01	20.37	1.193

[1] In precipitation solution.
[2] In precipitate.

Fig. 1 shows that the ratio P/Ca in the salt is constant and corresponds to the formula $C_6H_8O_{24}P_6Ca_5$ when precipitation takes place in the p_H-interval $4.6 < p_H < 6.9$. Outside this interval

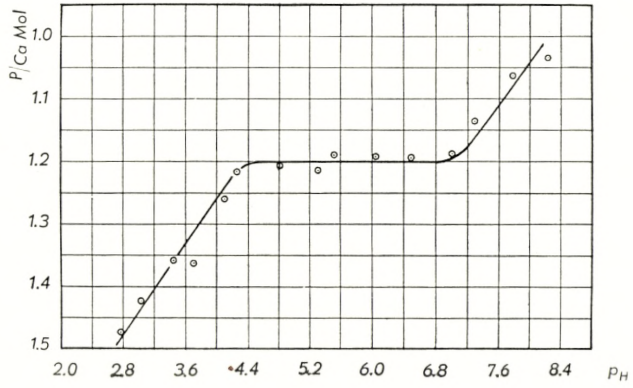


Fig. 1. Correlation between p_H in the solution and the ratio P/Ca in the precipitated salt.

the composition of the salt changes. The precipitation of other salts with a simple P/Ca ratio has failed.

As the pentacalcium phytate precipitate is fairly easily filtered off and as its composition is constant in a fairly large p_H-interval, it was to be expected that the salt was crystalline. This, however, is not the case. Mr. A. Tovborg Jensen has kindly pre-

pared X-ray powder diagrams of a series of precipitations, precipitated under various conditions. They were all amorphous. An example is shown in Fig. 2.

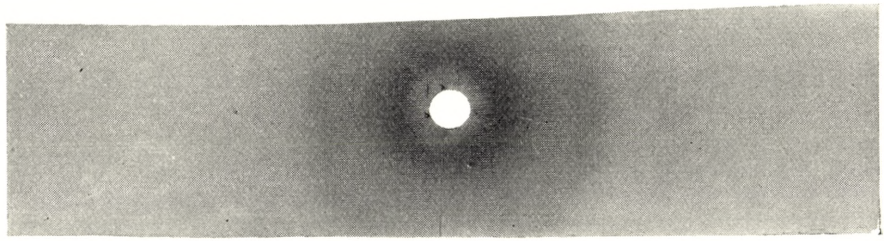


Fig. 2. X-ray powder diagram of pentacalcium phytate precipitated at p_H 5. Experimental conditions: Camera diameter 57,4 mm. Co-radiation, Fe-filter. Exposure 6 hours, pinhole diameter 0.6 mm. The two diffraction rings by registration with the Zeiss Photometer were found to correspond to glancing angles of 5°.7 and 16°.4.

2. Determination of the Dissociation Constants of Phytic Acid in 0.2 M and 1.0 M NaCl.

The dissociation constants have been determined by measurements with the H_2-electrode in 0.2 and 1.0 M sodium chloride solutions. The electrode vessel used was one constructed by K. J. PEDERSEN (1936); it consists of two Jena glass filters through which the hydrogen was passed into the solution. The bridge solution was 3.5 M potassium chloride. The hydrogen was taken from a steel flask and was washed in pyrogallol-potassium hydroxide and in a solution of sodium chloride of the same concentration as that to be examined. All readings were made at a temperature of 37° C ± 0°.1 C. Potentiometer: Wolff. Sensitivity of galvanometer: 2 mm. for 0.01 millivolt. The reference electrode was a hydrogen electrode in a solution which was 0.01 M in HCl and 0.2 and 1.0 M, respectively, in NaCl.

Preparation of Solutions.

10 ml. 0.01 M sodium phytate, 5 or 25 ml. 4.0 M sodium chloride and the amount of 0.02 N HCl entered in the tables was pipetted into a 100 ml. volumetric flask. The flask was filled to the mark with CO_2-free water. The figures given are the averages of two readings differing 0.2 millivolt at most.

$-\log[H^+]$ was calculated from the voltage difference π by the equation: $-\log[H^+] = 2.00 + \frac{\pi}{0.0615}$; $-\log[OH^-]$ was calculated from: $p_{K_c} = -\log[H^+] - \log[OH^-]$, where $p_{K_c} = 13.38$ in 1 M NaC and $p_{K_c} = 13.57$ in 0.2 M NaCl (BJERRUM and UNMACK (1929)). $\bar{n} = \frac{C_{HCl} - [H^+] + [OH^-]}{C_{phytate}}$ represents the average number of acidic hydrogen atoms of the phytate molecule at the p_H in question.

Table 3.

$C_{phytate} = 10^{-3}$ M; $C_{NaCl} = 0.2$ M; Reference Electrode: 0.01 N HCl, 0.2 M NaCl; 37° C.

$C_{HCl} \cdot 10^{-3}$	π Volt	$-\log[H^+]$	$-\log[OH^-]$	$\bar{n}$
0	0.5012	10.15	3.42	0.38
0.5	0.4914	9.99	3.58	0.76
1.5	0.4569	9.43	4.16	1.56
2.5	0.4193	8.82	4.75	2.52
3.5	0.3623	7.89	5.68	3.50
4.5	0.2696	6.38	7.21	4.50
5.5	0.1916	5.12	—	5.49
6.5	0.1017	3.65	—	6.26
7.5	0.0647	3.05	—	6.61
8.5	0.0486	2.79	—	6.88
9.5	0.0373	2.61	—	7.04
10.5	0.0292	2.47	—	7.11
11.5	0.0231	2.38	—	7.33
12	0.0201	2.33	—	7.32

$-\log[H^+]$ for $\bar{n} = 7.5$, 6.5, 5.5, 4.5, 3.5, 2.5, 1.5 and 0.5 was read by graphical interpolation (see Fig. 3) from the corresponding values of $\bar{n}$ and $-\log[H^+]$, which have been given in Table 3:

$\bar{n} =$	7.5	6.5	5.5	4.5	3.5	2.5	1.5	0.5
$-\log[H^+] =$	2.20	3.22	5.10	6.42	7.85	8.80	9.48	10.13

These values of $-\log[H^+]$ form a preliminary approximation to eight of the twelve dissociation constants of phytic acid.

Table 3 shows that determination of the remaining four preliminary constants is impossible, as this would require knowledge of $-\log[H^+]$ from $\bar{n} = 7.5$ to $\bar{n} = 12$.

Table 4.

$C_{phytate} = 10^{-3}$ M; $C_{NaCl} = 1.0$ M; Reference Electrode: 0.01 n HCl, 1.0 M NaCl; 37° C.

$C_{HCl} \cdot 10^{-3}$	π Volt	$-\log [H^+]$	$-\log [OH^-]$	$\bar{n}$
0	0.4631	9.53	3.85	0.14
0.5	0.4446	9.23	4.15	0.57
1.5	0.4096	8.66	4.72	1.52
2.5	0.3764	8.12	5.26	2.51
3.5	0.3255	7.29	—	3.50
4.5	0.2333	5.79	—	4.50
5.5	0.1585	4.58	—	5.47
6.5	0.0902	3.47	—	6.16
7.5	0.0587	2.95	—	6.38
8.5	0.0437	2.71	—	6.55
9.5	0.0335	2.54	—	6.62
10.5	0.0262	2.43	—	6.78
11.5	0.0203	2.33	—	6.82
12	0.0179	2.29	—	6.87

Table 5.

$C_{phytate} = 10^{-2}$ M; $C_{NaCl} = 1.0$ M; Reference Electrode: 0.1 M HCl, 1.0 M NaCl; 37° C.

$C_{HCl} \cdot 10^{-2}$	π Volt	$-\log [H^+]$	$-\log [OH^-]$	$\bar{n}$
0	0.5352	9.70	3.68	0.02
0.5	0.5176	9.42	3.96	0.51
1.5	0.4872	8.92	4.46	1.50
2.5	0.4477	8.28	5.13	2.50
3.5	0.3841	7.25	—	3.50
4.5	0.2904	5.72	—	4.50
5.5	0.2151	4.50	—	5.50
6.5	0.1242	3.02	—	6.40
7.5	0.0763	2.24	—	6.92
8.5	0.0567	1.92	—	7.30
9.5	0.0437	1.71	—	7.55
10.5	0.0345	1.56	—	7.75
11.5	0.0273	1.44	—	7.87
12	0.0242	1.39	—	7.93

Exactly similar measurements were made in 1.0 M sodium chloride. The results have been entered in Table 4.

Table 4 shows that corresponding values of $\bar{n}$ and $-\log[H^+]$ only allow determination of seven out of the twelve dissociation constants of phytic acid. Determination of another one or two constants was attempted by making a series of measurements in a ten times stronger concentration of phytic acid. The results of the measurements have been given in Table 5.

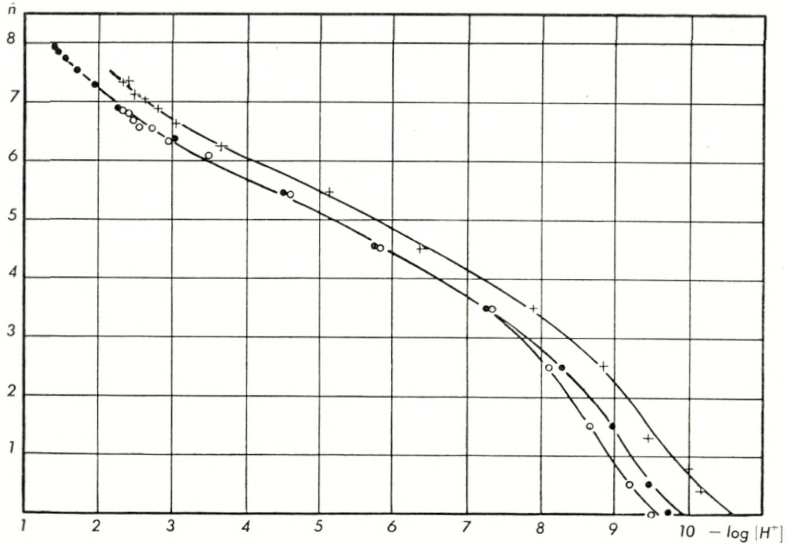


Fig. 3. Corrected titration curve for phytic acid.
+——+ $C_{phytate} = 10^{-3}$; $C_{Na\,Cl} = 0.2$
●——● - $= 10^{-2}$; - $= 1.0$
○——○ - $= 10^{-3}$; - $= 1.0$

When corresponding values of $\bar{n}$ and $-\log[H^+]$ are plotted in a diagram, we see that the curves from Table 4 and Table 5 are identical for values of $\bar{n}$ larger than 3.5, i. e. in the acid and neutral region. The values of $-\log[H^+]$ corresponding to n = 7.5, 6.5, 5.5, 4.5, and 3.5 have therefore been read from the joint curve, the values for n = 2.5, 1.5 and 0.5 from the curve plotted from Table 4 (0.001 M phytate). In this way preliminary values were found for the eight dissociation constants in 1.0 M NaCl:

$\bar{n} =$	7.5	6.5	5.5	4.5	3.5	2.5	1.5	0.5
$-\log[H^+] =$	1.75	2.77	4.53	5.76	7.28	8.12	8.68	9.25

From the corresponding values of $\bar{n}$ and $-\log[H^+]$ it is possible to calculate a set of dissociation constants which mutually agree. The calculation may be carried out in several ways. In the present paper the procedure worked out by J. BJERRUM (1941) for complexity constants has been adapted to dissociation constants; according to this method the constants are computed by successive approximation of the preliminary constants, according to the following formula:

$$(1) \quad K_n = [H^+]_{N-\left(n-\frac{1}{2}\right)} \cdot \frac{1+\sum_{t=1}^{t=n-1} \frac{(1+2t)\,[H^+]^t}{K_{n-1} \cdot K_{n-2} \cdots K_{n-t}}}{1+\sum_{t=1}^{t=N-n} \frac{(1+2t)\,K_{n+1} \cdot K_{n+2} \cdot \cdots \cdot K_{n+t}}{[H^+]^t}}$$

K_n is the n^{th} of the n consecutive dissociation constants. N is the total number of dissociation constants = 12, and t is a parameter which must take all integer values between the fixed limits. The terms dealing with one or more of the four unknown preliminary dissociation constants have been disregarded. The following two sets of dissociation constants were determined after three successive approximations according to the above formula (K_5 has not been corrected, as the correction would be unilateral):

	$-\log K_5$	$-\log K_6$	$-\log K_7$	$-\log K_8$	$-\log K_9$	$-\log K_{10}$	$-\log K_{11}$	$-\log K_{12}$
In 1.0 M NaCl...	1.75	2.68	4.59	5.71	7.34	8.19	8.78	8.95
In 0.2 M NaCl...	2.20	3.24	5.15	6.39	7.96	8.82	9.61	9.83

In order to calculate the solubility product of pentacalcium phytate it is neccessary to know the concentration of the phytate ion with ten negative charges. The total phytate concentration may be determined analytically, and the ratio (phytate ion with ten charges)/(total phytate concentration) may be calculated from the dissociation constants.

$[Py_0]$ represents the concentration of non-dissociated phytic acid, and $[Py_n]$ the concentration of phytate ions with n negative charges.

$$[Py_t] = [Py_0] + [Py_1] + \cdots\cdots [Py_{12}]$$

from the dissociation equations we have:

$$[Py_n] = K_1 \cdot K_2 \cdots\cdots K_n \cdot \frac{1}{[H^+]^n} \cdot [Py_0],$$

and from this:

$$(2) \qquad \frac{[Py_{10}]}{[Py_t]} = \frac{K_1 \cdot K_2 \cdots\cdots K_{10} \cdot \frac{1}{[H^+]^{10}} \cdot [Py_0]}{[Py_0] + K_1 \cdot \frac{1}{[H^+]} \cdot [Py_0] + \cdots + K_1 \cdot K_2 \cdots K_{12} \cdot \frac{1}{[H^+]^{12}} \cdot [Py_0]}.$$

If we put $K_1 = K_2 = K_3 = K_4 = K_5$, a simple substitution of the values determined for $K_5 \cdots\cdots K_{12}$ shows that if only $-\log[H^+]$ is larger than 4, the four first constants, which we have not been able to determine, may be disregarded. In this special case the formula may therefore be written

$$(3) \qquad \frac{[Py_{10}]}{[Py_t]} = \frac{K_5 \cdot K_6 \cdot K_7 \cdot \cdots\cdots K_{10} \cdot \frac{1}{[H^+]^{10}}}{\frac{1}{[H^+]^4} + \frac{K_5}{[H^+]^5} + \frac{K_5 \cdot K_6}{[H^+]^6} + \cdots + \frac{K_5 \cdot K_6 \cdots K_{12}}{[H^+]^{12}}}.$$

Corresponding to the values of $-\log[H^+]$, at which the solubility of pentacalcium phytate has been determined experimentally, Py_{10}/Py_t has been calculated by this formula. The results have been given in Tables 8 and 9.

3. The Solubility of Pentacalcium Phytate in 0.2 and 1.0 M Sodium Chloride.

The calcium phytate used for solubility determinations was prepared as described on p. 5; precipitation took place at p_H 5, and the salt was dried at room temperature. Before analysis the preparation was dried in vacuo over P_2O_5 at 105° C. The water content after drying in this manner was determined by further drying at 200° C. in vacuo over P_2O_5.

	C	H	P	Ca	H_2O	Ash	P/Ca
Found per cent.	8.08	1.78	20.68	22.06	6.14	71.3	1.210
Calculated for $C_6H_8O_{24}P_6Ca_5$, $3\,H_2O$ per cent.	7.97	1.55	20.58	22.12	5.87	—	1.200

5 or 25 ml. 4.0 M sodium chloride were measured into a 100 ml. volumetric flask and a suitable quantity of 0.1 M HCl or NaOH was added; the flask was filled to the mark with CO_2-free water. About 1 grm. calcium phytate was added and the flask was rotated for about 24 hours at 37° C. ± 0.1° C. Before samples were taken, the flask was left to stand in the thermostat for about one hour in order to let the precipitate set. The desired quantity of the clear supernatant fluid was sucked off through a filterstick G 3.

Analytical Methods.

Calcium was determined by the methods of Larson and Greenberg (1938) as oxalate by oxidation with ceri sulphate and titration with ferrous ammonium sulphate, using ferroin as indicator. This method is more accurate than the usual titration with potassium permanganate. 10 determinations of 1 mg. calcium show a standard deviation of 0.8 per cent.

Phosphorus was determined according to Widmark and Wahlquist (1931). The organic matter is ashed with sulphuric acid and nitric acid and the phosphorus precipitated as ammonium phosphorus molybdate, which is titrated with sodium hydroxide. 10 determinations of 0.5 mg. phosphorus show a standard deviation of 1.2 per cent.

The hydrogen ion concentration was determined with the glass electrode. 0.01 M HCl in 0.2 or 1.0 M sodium chloride was used as standard solution ($-\log[H^+] = 2.00$).

The Solubility is Independent of the Quantity of Solid Calcium Phytate Present.

a. A suspension of 0.2 grm. calcium phytate in 100 ml. 1.0 M NaCl, containing 1.5 ml. 0.1 N HCl.

b. A suspension of 2.0 grm. calcium phytate in 100 ml. of the same solution.

The following values were found after rotation at 37° C. for 24 hours:

	a	b
$-\log [H^+]$	5.37	5.38
C_{Ca} mM per l.	5.98	6.02

Connection between Solubility and Temperature.

Fig. 4 shows that the solubility of calcium phytate decreases rapidly as the temperature increases.

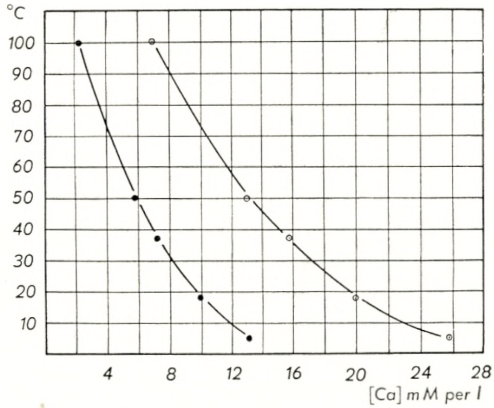


Fig. 4. The solubility of calcium phytate at various temperatures.
○——○ In 1 M NaCl
●——● In 0.2 M NaCl

Reversibility of Solubility.

The solubility of calcium phytate was determined in the following solutions:

a. 0.2 M sodium acetate, 0.15 M acetic acid.

b. 0.2 M sodium acetate, 0.15 M acetic acid, 0.8 M sodium chloride.

The solubility was first determined at 37° C.; the temperature was then lowered to 18° C., and the solubility determined at this temperature. The temperature was again raised to 37° C., so that the equilibrium now was approached from the supersaturated side. The following results show that the same values of solubility were reached from the supersaturated as from the undersaturated side:

		a	b
C_{Ca} mM per l.,	37° C.	7.22	15.80
—	18° C.	9.85	20.00
—	37° C.	7.27	15.75

Connection between Solubility and Hydrogen Ion Concentration.

Table 6.
The Solubility of Pentacalcium Phytate in 1.0 M NaCl at 37° C.

$-\log [H^+]$	C_{Ca} m M per l.	C_P m M per l.	P/Ca Mol
4.20	37.8	—	—
4.52	22.5	25.8	1.15
4.74	15.05	—	—
5.02	9.05	10.70	1.18
5.40	5.85	—	—
5.89	3.47	4.12	1.19
6.30	2.48	—	—
6.62	1.86	2.18	1.17
7.03	1.18	1.48	1.25
7.64	0.685	—	—

The tables show that in the interval $5 < -\log [H^+] < 7$ the ratio P/Ca is the same in the solution as in the salt.

Tab e 7.
The Solubility of Pentacalcium Phytate in 0.2 M NaCl at 37° C.

$-\log [H^+]$	C_{Ca} m M per l.	P_P m M per l.	P/Ca Mol
4.15	28.9	30.0	1.04
4.57	14.25	—	—
4.81	8.25	—	—
5.03	5.45	6.32	1.16
5.44	3.10	—	—
5.87	1.65	2.02	1.22
6.24	1.03	—	—
6.70	0.595	0.693	1.17
7.05	0.320	—	—
7.43	0.185	—	—

Calculation of the Solubility Product of Pentacalcium Phytate in 0.2 M and 1.0 M NaCl.

The solubility product $[Ca]^5 \cdot [Py_{10}] = L$ has been calculated as follows: [Ca] was taken from Tables 6 and 7; $[Py_{10}]/[Py_t]$

was calculated from the formula (3); $[Py_t]$ was calculated from: $5 \cdot [Py_t] = [Ca]$.

Table 8.
Calculation of the Solubility Product of Pentacalcium Phytate in 1 M NaCl at 37° C.

$-\log [H^+]$	4.20	4.52	4.74	5.02	5.40	5.89	6.30	6.62	7.03	7.64
$-\log \frac{[Py_{10}]}{[Py_t]}$	9.216	8.059	7.283	6.379	5.256	3.987	3.127	2.409	1.663	0.807
$-\log [Ca]$	1.423	1.648	1.823	2.043	2.233	2.460	2.606	2.731	2.928	3.164
$-\log [Py_t]$	2.122	2.347	2.522	2.742	2.932	3.159	3.305	3.430	3.627	3.863
$-\log [Py_{10}]$	11.338	10.406	9.805	9.121	8.188	7.146	6.432	5.839	5.290	4.670
$-\log L$	18.453	18.646	18.920	**19.336**	**19.353**	**19.446**	**19.462**	**19.494**	19.930	20.490

The table shows that the solubility product in 1.0 M NaCl is almost constant in the interval $5.02 < -\log [H^+] < 6.62$. In this interval the average of our five determinations was:

$$-\log L = 19.4 \ (1.0\,M\ NaCl)$$

The solubility product has been determined in the same manner in 0.2 M NaCl.

The calculations have been entered in Table 9.

Table 9.
Calculation of the Solubility Product of Pentacalcium Phytate in 0.2 M NaCl at 37° C.

$-\log [H^+]$	4.15	4.57	4.81	5.03	5.44	5.87	6.24	6.70	7.05	7.43
$-\log \frac{[Py_{10}]}{[Py_t]}$	11.797	10.152	9.245	8.448	7.057	5.724	4.697	3.563	2.800	2.056
$-\log [Ca]$	1.539	1.846	2.084	2.264	2.509	2.783	2.987	3.226	3.495	3.733
$-\log [Py_{10}]$	2.238	2.545	2.783	2.963	3.208	3.482	3.686	3.925	4.194	4.432
$-\log [Py_t]$	14.035	12.697	12.028	11.411	10.265	9.206	8.383	7.488	6.994	6.488
$-\log L$	21.730	21.927	22.448	**22.731**	**22.810**	**23.121**	**23.318**	**23.618**	24.469	25.153

The values of $-\log L$ are not quite so constant as in 1.0 M NaCl. In the interval $5.03 < -\log [H^+] < 6.70$ the average of five determinations was:

$$-\log L = 23.0 \ (0.2\,M\ NaCl).$$

4. Formation of Calcium Phytate Complex Ions.

The experiments described on p. 5 show that solutions of calcium phytate are considerably more acid than the corresponding solutions of sodium phytate. Similar relations are found in solutions of calcium phosphate and are explained by a pronounced acidity of the calcium complexes formed (N. Bjerrum et al. (1936)). They state that in solutions of calcium phosphates the complex formation is usually less than 10 per cent. The experiments described above, however, render it likely that in solutions of calcium phytate a considerably larger part of the dissolved calcium is found as a calcium phytate complex ion. Like precipitated calcium, complex bound calcium is probably not absorbed at all from the intestine, and we have therefore tried to determine the degree of complexity in solutions of calcium phytate.

In order to examine this, solutions with varying contents of calcium and phytate were rotated with solid calcium iodate (Kilde (1934)). From the solubility product of calcium iodate: $[Ca^{++}] \cdot [JO_3^-]^2 = K$, the concentration of calcium ions may be calculated from the iodate concentration. The total quantity of dissolved calcium was determined in the usual manner. The fact that the solubility of calcium iodate is fairly large as compared with that of calcium phytate presents a difficulty, as it is necessary to avoid precipitation of calcium phytate. The experiments therefore can only be carried out within a fairly limited interval of the concentrations of hydrogen ions, phytate and calcium ions. Contrary to calcium iodate, calcium phytate has its greatest solubility at low temperatures. The principal experiments therefore have been made at 18° C., instead of at 37° C.

Table 10 gives the results of the complexity determinations made at 18° C., $p_H = 4.3$ and μ about 0.2. The solubility product of the calcium iodate has been calculated from the average of the iodate analyses in the last two experiments. The calcium ion concentration has been calculated from the equation: $[Ca^{++}] = \frac{K}{[JO_3^-]^2}$.

Table 11 gives the results of a similar series of experiments at μ about 1.

Table 10.

Solution A: 0.02 M sodium phytate, 0.12 M HCl.

Solution B: 0.05 M calcium nitrate, 0.07 M sodium acetate, 0.14 M acetic acid.

Solution C: 0.1 M sodium acetate, 0.12 M sodium chloride, 0.2 M acetic acid.

18° C; p_H 4.3.

$[Ca^{++}] \cdot [JO_3^-]^2 = K = 3.00 \cdot 10^{-6}$ ($\mu = 0.247$)

ml. A	ml. B	ml. C	$C_{phytate}$ m M per l.	$C_{Ca_{total}}$ m M per l.	$C_{JO_3^-}$ m M per l.	C_{Ca++} m M per l.	$C_{Ca_{complex}}$ m M per l.	$\frac{C_{Ca_{complex}}}{C_{phytate}}$
50	0	0	20.00	12.78	30.90	3.14	9.64	0.48
25	10	0	14.28	22.05	19.45	7.93	14.12	0.97
25	25	0	10.00	29.77	13.67	16.06	13.71	1.37
10	25	0	5.71	39.80	9.93	30.41	9.39	1.65
10	40	0	4.00	43.75	9.05	36.62	7.13	1.78
5	45	0	2.00	48.55	8.18	44.80	3.75	1.88
0	0	50	0	9.20	18.16	(9.08)	—	—
0	0	50	0	9.25	18.20	(9.10)	—	—

In order to examine the connection between complex formation and p_H, a short series of experiments was made at $p_H = 5$

Table 11.

Solution A, B, and C as in Table 10, but with 0.78 M NaCl added pr l.

18° C; $p_H = 4.3$.

$[Ca^{++}] \cdot [JO_3^-]^2 = K = 10.2 \cdot 10^{-6}$ ($\mu = 1.041$)

ml. A	ml. B	ml. C	$C_{phytate}$ m M per l.	$C_{Ca_{total}}$ m M per l.	$C_{JO_3^-}$ m M per l.	C_{Ca++} m M per l.	$C_{Ca_{complex}}$ m M per l.	$\frac{C_{Ca_{complex}}}{C_{phytate}}$
50	0	0	20.00	14.40	33.30	9.19	5.21	0.26
20	10	0	13.33	26.60	23.20	18.95	7.65	0.57
15	15	0	10.00	33.58	19.75	26.22	7.36	0.74
10	20	0	6.67	41.75	17.02	35.18	6.57	0.99
5	20	0	4.00	48.50	15.20	44.12	4.38	1.09
3	20	0	2.61	52.30	14.40	49.15	3.15	1.20
0	0	50	0	13.80	27.30	(13.65)	—	—
0	0	50	0	13.85	27.40	(13.70)	—	—

and μ about 0.2. On account of the solubilities of the salt in question the investigation cannot be made at higher p_H.

Table 12.

Solution A: 0.02 M sodium phytate, 0.01 M HCl, 0.01 M NaCl.

Solution B: 0.05 M calcium nitrate, 0.07 M sodium acetate, 0.35 M acetic acid.

Solution C: 0.10 M sodium acetate, 0.12 M sodium chloride, 0.2 M acetic acid.

18° C; $p_H = 5.0$

$[Ca^{++}] \cdot [JO_3^-]^2 = K = 3.08 \cdot 10^{-6}$ ($\mu = 0.248$)

ml. A	ml. B	ml. C	$C_{phytate}$ m M per l.	$C_{Ca_{total}}$ m M per l.	$C_{JO_3^-}$ m M per l.	C_{Ca++} m M per l.	$C_{Ca_{complex}}$ m M per l.	$\frac{C_{Ca_{complex}}}{C_{phytate}}$
10	0	40	4.0	11.03	22.55	6.06	4.97	1.24
10	10	30	4.0	17.35	16.74	11.00	6.35	1.59
5	10	35	2.0	17.05	15.08	13.55	3.50	1.75
0	0	50	0	9.25	18.36	(9.18)	—	—

In order to find the connection between complex formation and temperature, experiments were also made at 30° C. At temperatures higher than 32° C. calcium iodate is decomposed.

Table 13.

Solution A, B and C as in Table 12.

30°C. $p_H = 4.3$.

$[Ca^{++}] \cdot [JO_3^-]^2 = K = 11.6 \cdot 10^{-6}$ ($\mu = 0.263$)

ml. A	ml. C	ml. C	$C_{phytate}$ m M per l.	$C_{Ca_{total}}$ m M per l.	$C_{JO_3^-}$ m M per l.	C_{Ca++} m M per l.	$C_{Ca_{complex}}$ m M per l.	$\frac{C_{Ca_{complex}}}{C_{phytate}}$
20	0	10	13.33	19.16	40.08	7.21	11.95	0.90
10	10	10	6.67	28.50	25.05	18.49	10.01	1.51
10	15	10	5.71	33.00	21.78	24.25	8.80	1.54
0	0	30	0	14.05	28.50	(14.25)	—	—

In Fig. 5 a graph has been plotted, showing the connection between $\bar{n} = \frac{C_{Ca_{complex}}}{C_{phytate}}$ and $-\log [Ca^{++}]$. The graph shows how many calcium atoms are on an average bound in the

Table 14.

Solution A, B and C as in Table 11.

30° C; $p_H = 4.3$.

$[Ca^{++}] \cdot [JO_3^-]^2 = K = 38.7 \cdot 10^{-6}$ ($\mu = 1.064$)

ml. A	ml. B	ml. C	$C_{phytate}$ m M per l.	$C_{Ca_{total}}$ m M per l.	$C_{JO_3^-}$ m M per l.	C_{Ca++} m M per l.	$C_{Ca_{complex}}$ m M per l.	$\frac{C_{Ca_{complex}}}{C_{phytate}}$
20	0	0	20	25.50	50.79	15.02	10.48	0.52
10	5	15	6.67	28.42	40.55	23.55	4.97	0.74
10	15	5	6.67	41.10	33.35	34.78	6.32	0.95
0	0	30	0	21.35	42.60	(21.30)	—	—

calcium phytate complex ion at a certain concentration of calcium ions.

Fig. 5 shows that the degree of complexity changes only slightly with variation in temperature; on the other hand it decreases with increasing ionic strength and increases with in-

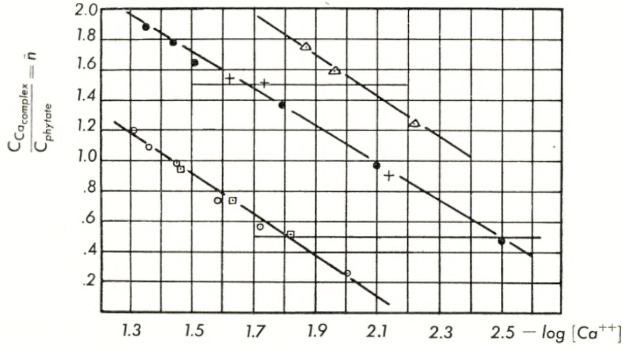


Fig. 5. Formation curves for calcium phytate complex ion.

● ——— ● 18° C. $p_H = 4.3$, μ about 0.2
○ ——— ○ 18° C. $p_H = 4.3$, - - 1
△ ——— △ 18° C. $p_H = 5.0$, - - 0.2
\+ ——— \+ 30° C. $p_H = 4.3$, - - 0.2
□ ——— □ 30° C. $p_H = 4.3$, - - 1

creasing p_H. This explains that the calculated solubility product (Tables 6 and 7) decreases as p_H increases.

Proceeding as we did for the dissociation constants (see Fig. 3) we may derive approximate values for the first two complexity constants, K_1 and K_2, from the values of $-\log [Ca^{++}]$, which correspond to $\bar{n} = 0.5$ and $\bar{n} = 1.5$;

	$-\log K_1$	$-\log K_2$
$p_H = 4.3$, μ about 0.2	2.50	1.67
$p_H = 4.3$, μ - 1	1.83	—
$p_H = 5.0$, μ - 0.2	—	2.04

From the results in Table 11 it can be calculated that in saturated solutions of calcium phytate, at p_H about 5 and μ about 3, approximately 0.20 per cent. of the dissolved calcium will be complex bound.

5. Comparative Investigations on the Solubility of Calcium Phytate and Some Calcium Phosphates.

In the p_H-interval which is most important as far as precipitation of calcium in the intestine is concerned, hydroxyl apatite, $Ca_5(PO_4)_3OH$, is the least soluble of the calcium phosphates. BJERRUM et al. (*l. c.*) have determined the solubility of hydroxyl apatite in much diluted salt solutions; their investigations thus are not exactly comparable with the measurements of the solubility of calcium phytate described above. The author therefore has determined the solubility of hydroxyl apatite in 0.2 M sodium chloride solutions in the same manner as described for calcium phytate, the hydroxyl apatite being precipitated *ad modum* BJERRUM et al. (*l. c.*). The solubility of secondary calcium phosphate, $CaHPO_4$, $2\,H_2O$, has also been measured in 0.2 M NaCl. The results of these measurements are shown in Fig. 6.

Fig. 6 shows that when equilibrium is approached from the under-saturated side, the calcium concentration in the saturated solution of hydroxyl apatite is slightly below the calcium concentration in the saturated solution of calcium phytate. Thus, if the calcium phosphate is precipitated in the intestine as hydroxyl apatite, there seems to be no reason to assume that calcium absorption should be more impaired by the presence of phytic acid in the food than by the presence of phosphate.

This mode of reasoning, however, does not consider the fact that when the acid contents of the stomach enter the intestine the solubility equilibrium is approached from the supersaturated side. In order to examine whether this is of any importance the following experiments were made:

a. A solution of hydroxyl apatite was made by rotating solid hydroxyl apatite in a solution which was 0.2 M in NaCl and 0.07 M in HCl; quantities of this solution sufficient to dissolve all the hydroxyl apatite were used. The solution obtained was 0.067 M in Ca and $p_H = 3.5$.

b. A solution of calcium phytate was made in the same manner; it was 0.074 M in Ca and $p_H = 3.4$.

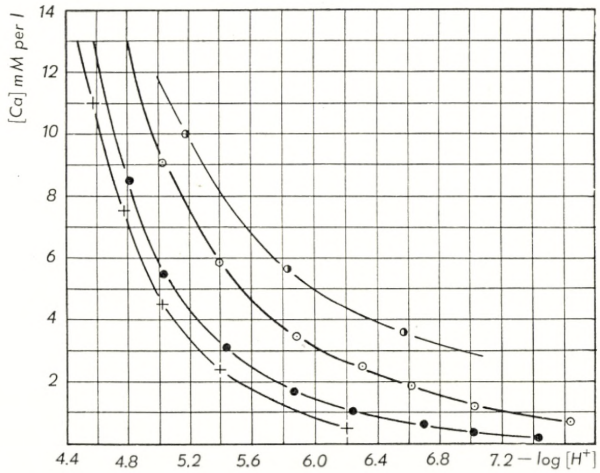


Fig. 6. Solubility of calcium phytate, hydroxyl apatite, and secondary calcium phosphate.

⊙——⊙ Calcium phytate in 1 M NaCl
●——● Calcium phytate in 0.2 M NaCl
+——+ Hydroxyl apatite in 0.2 M NaCl
◑——◑ Secondary calcium phosphate in 02 M NaCl.

Each of these solutions was mixed at a temperature of 37° C. with equal parts of a solution which was 0.2 M in sodium acetate and 0.02 M in acetic acid. Two 100 ml. samples were taken from each of these mixtures, making four samples in all. 1 grm. hydroxyl apatite was added to one of the hydroxyl apatite samples, and 1 grm. calcium phytate to one of the phytate samples. All four samples were rotated at 37° C. In each experiment the quantities of calcium dissolved at different points of time were determined. The results have been plotted in Fig. 7.

Fig. 7 shows that whether a supersaturated solution of calcium phytate contains solid calcium phytate or not, the equilibrium is reached instantly. In the solution of hydroxyl apatite,

calcium is precipitated slowly when solid hydroxyl apatite is present. The solution of hydroxyl apatite, which did not contain solid hydroxyl apatite, did not show turbidity until about

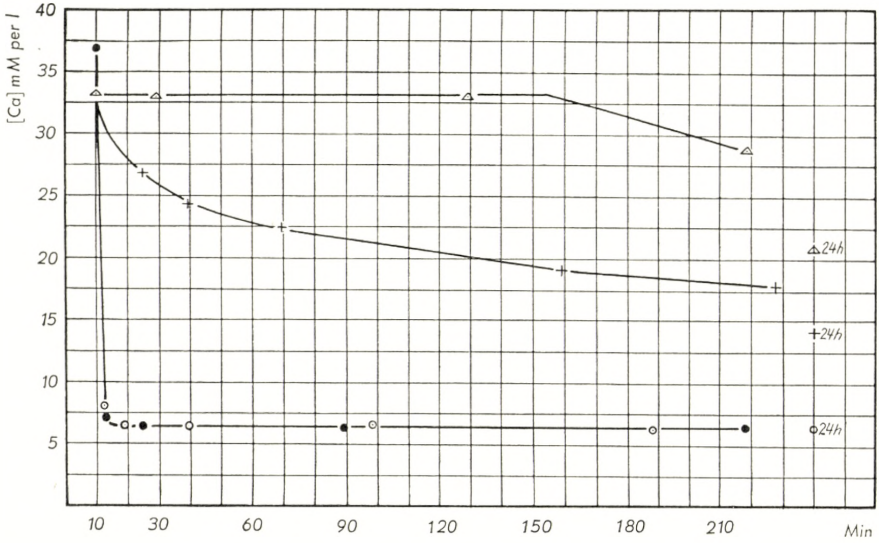


Fig. 7. Precipitation of calcium in solutions of calcium phytate and hydroxyl apatite.

⊙——⊙ Calcium phytate at p_H 5.08
●——● - - with solid calcium phytate at p_H 5.04
△——△ Hydroxyl apatite at p_H 5.28
+——+ - - with solid hydroxyl apatite at p_H 5.20.

two hours had passed. After 24 hours the sediment was clearly crystalline. The X-ray powder diagram (A. Tovborg Jensen) together with the analyses given below showed that the sediment was secondary calcium phosphate.

	Ca	P	Loss at 600° C.
Found per cent.....	23.32	18.25	26.15
Calculated for $CaHPO_4$, $2H_2O$, per cent....	23.50	18.12	26.15

In a series of experiments with varying contents of calcium and phosphate in diluted acid solutions, which were brought to a p_H about 5 at 37° C., the precipitate formed likewise consisted of crystalline secondary calcium phosphate.

These experiments seem to indicate that when the acid stomach contents containing calcium and phosphate are emptied

into the intestine, a precipitate of secondary calcium phosphate will form. The precipitation, however, is slow. According to the present investigations it therefore seems probable that the presence of phytic acid in the food reduces the concentration of calcium ions in the intestine considerably. This is due to the fact that calcium phytate is only slightly soluble, and particularly to the fact that calcium phytate is not liable to form supersaturated solutions. It is moreover of importance that a considerable part of the dissolved calcium will be complex bound in the presence of phytic acid.

Summary.

(1) In solutions of phytic acid and calcium ions, amorphous pentacalcium phytate precipitates in the p_H-interval $5 < p_H < 7$.

(2) Eight of the dissociation constants of phytic acid have been determined in 0.2 and 1.0 M NaCl.

(3) The solubility of pentacalcium phytate has been determined at various hydrogen ion concentrations in 0.2 and 1.0 M NaCl, respectively.

(4) The solubility product of pentacalcium phytate (complex formation having been disregarded) was calculated to be $10^{-19.4}$ in 1.0 M NaCl and $10^{-23.0}$ in 0.2 M NaCl in the interval $5 < -\log H^+ < 6.6$.

(5) The formation of calcium phytate complex ions has been examined.

(6) The calcium phosphate precipitated in the intestine is probably $CaHPO_4$, $2\,H_2O$.

(7) Pentacalcium phytate is less soluble than secondary calcium phosphate. The former salt is precipitated instantly, whilst the latter precipitates slowly.

(8) Calcium absorption is more likely to be impaired when the food contains large quantities of phytic acids than when it contains a surplus of phosphate. The present investigations thus support the theory of HARRISON and MELLANBY (*l. c.*).

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