XXI. STUDIES ON THE METABOLISM OF CALCIUM AND PHOSPHORUS AND ON THE AVAILABILITY OF THESE ELEMENTS FROM MILK AND FROM AN INORGANIC SOURCE

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SOME time ago experiments were reported from this laboratory [Henry & Kon, 1937, 1] on the availability to rats of Ca and P in raw and pasteurized milks. While no difference was found in this respect between the two milks two rather puzzling observations were made. In the first place, though the rats received only 60 to 70 % of the Ca and P normally retained by rats of their age on an adequate diet, they utilized only some 80 % of their intake of these minerals. Secondly, 90 % of the excreted Ca was found in the urine, a result hardly to be expected as it is usually believed that Ca is largely excreted in the faeces.

We have since carried out further experiments to determine whether this unusual excretion of Ca via the kidney and the rather low retention were due to the quality or to the quantity of the dietary Ca or possibly also to a relative shortage of P. At the same time we investigated the possibility of increasing the retention of P. It should be recalled that Ellis & Mitchell [1933] and Fairbanks & Mitchell [1936] had obtained almost 100 % retentions of Ca from diets low in Ca but carrying a generous supply of P.

In these experiments the level, ratio and source of Ca and P were varied. Milk as a source of these elements was compared with Ca phosphate. Unless otherwise stated the general experimental methods were as described in detail by Henry & Kon [1937, 1].

PRELIMINARY EXPERIMENTS

*Experimental.*¹ These were exploratory experiments which dealt mainly with factors affecting the assimilation of Ca. In one experiment Ca was supplied from milk and from $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ at levels below the normal retention, in each case either with an equally subnormal level of P or with a more generous supply. In the second experiment the same plan was followed but all levels of intake of Ca and of P were raised.

1. *The diets.* To the basal low Ca and P diet of Henry & Kon [1937, 1] (diet 64, Table I) several supplements were added: (a) raw milk; (b) an equivalent quantity of diet 75, which was made from diet 64 by the addition of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ (Table I); 18 g. of diet 75 were calculated to be equivalent in calorie, Ca and P contents to 100 ml. of the raw milk; (c) raw milk + added P, derived from $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$; (d) diet 75 + added P from the same source. This extra P was calculated to cover the urinary and faecal loss of P observed in the previous experiments [Henry & Kon, 1937, 1], which amounted to

¹ Read in part before the Biochemical Society, 9 October 1937 [Henry & Kon, 1937, 2].

0.21 mg. P for each ml. of milk intake. The addition did not suffice to bring the intake of P to the level normally retained by rats (Table IV). The various supplements were fed separately from the basal diet, milk in the liquid form (with or without added Na phosphate) and diet 75 mixed to a paste with $\frac{3}{4}$ of its weight of water. When Na phosphate was added to it, the solution replaced part of the water. Table I gives the composition and analyses of the diets.

Table I. *Exps. 1 and 2. Composition and analyses of diets and milk*

	Diet 64	Diet 75	Milk*
Egg white†	14.25	13.86	—
Butter fat	5.00	4.86	—
Maize starch	68.40	66.56	—
Castor sugar	9.50	9.24	—
Ca- and P-free salt mixture‡	2.85	2.77	—
CaHPO ₄ · 2H ₂ O	—	2.71	—
% Ca	0.02243	0.69460	0.12540
% P	0.02585	0.54060	0.10700
% Moisture	9.96	10.20	—

* Average of 8 bi-weekly analyses. There was very little variation between samples. The Ca and P figures are per 100 ml.

† Dried, heat-coagulated from new laid eggs.

‡ Henry & Kon [1937, 1].

18 g. of diet 75 contained 0.1252 g. Ca and 0.0973 g. P. The small discrepancy in the P content of diet 75 and of the equivalent volume of milk is explained by the fact that the P in the milk was slightly higher than anticipated. The vitamin supplements were as described elsewhere [Henry & Kon, 1937, 1].

2. *The animals.* 4 groups of 4 litter-mate male rats were used. The rats were 26–27 days old and weighed 59–71 g. at the beginning of the experiment. Litter-mates within each group were assigned by lot to the 4 treatments. They were placed in glass metabolism cages [Henry & Kon, 1937, 1]. The intakes of diet 64 and of milk or diet 75 were equalized between groups (a) and (b) and between groups (c) and (d) respectively. Table II shows that in Exp. 1 the food intakes of all 4 groups were very similar.

3. *Exp. 1.* As in previous work [Henry & Kon, 1937, 1] the milk and diet 75 were fed at such levels that the intakes of Ca and P from them were well below the normal retentions as defined by Sherman & Macleod [1925] and Sherman & Quinn [1926]. As already mentioned, groups (c) and (d) received additional P from a 6.3 % solution of Na₂HPO₄ · 12H₂O. After a preliminary period of 3 days on the experimental diets a collection period of 14 days was started. The excreta of each group of 4 rats were pooled for analysis.

4. *Exp. 2.* When Exp. 1 was finished the amounts of milk and diet 75 were so increased as slightly to exceed the calculated normal retentions. Groups (c) and (d) again received extra P from a 6.4 % solution of Na₂HPO₄ · 12H₂O. Otherwise the details were the same as in Exp. 1. The preliminary period lasted 4 days and was followed by a 14 days' collection period. The same rats were used in both experiments.

5. *Results.* Table II gives the food intakes, weights of the rats and the weight of faeces. The mean balance figures for Ca and P are given in Table III. Further data will be found in Table IV.

(a) *Exp. 1.* In Exp. 1 the behaviour of rats receiving milk as a source of Ca and P (group (a)) entirely confirmed the results previously reported [Henry & Kon, 1937, 1] regarding the retention of these minerals and their paths of excretion on subnormal intakes. Again only about 80 % of the ingested Ca and P were retained and over 90 % of the excreted Ca was in the urine. When the

Table II. *Exps. 1 and 2 (14 days each). Food intakes, weights of rats and of faeces*

Group and source of Ca and P	Food intake				Wt. of rats, g.		Wt. of faeces† g.
	Diet 64 g.	Milk ml.	Diet 75 g.	Na ₂ HPO ₄ solution ml.			
					Initial	Final	
(a) Milk	106.13	164.99	—	—	67.5	107.7	2.49
(b) Diet 75	106.11	—	29.69	—	65.8	106.0	2.16
(c) Milk + Na ₂ HPO ₄	108.68	165.57	—	6.619	67.5	109.2	2.62
(d) Diet 75 + Na ₂ HPO ₄	108.68	—	29.75	6.614	67.8	107.8	2.31
Exp. 2							
(a) Milk	107.32	293.48	—	—	117.0	150.8	3.80
(b) Diet 75	107.32	—	52.76	—	115.8	149.5	2.92
(c) Milk + Na ₂ HPO ₄ (i)*	100.69	324.75	—	12.99	121.3	151.0	3.87
Milk + Na ₂ HPO ₄ (ii)†	105.82	315.33	—	12.61	116.0	151.3	—
(d) Diet 75 + Na ₂ HPO ₄ (i)*	101.00	—	58.34	12.96	119.3	153.8	2.84
Diet 75 + Na ₂ HPO ₄ (ii)†	105.67	—	56.61	12.58	115.7	156.3	—

* (i) = Average for all 4 rats.

† (ii) = Average for 3 rats, omitting the rats mentioned in the text, p. 176.

‡ Air dry.

Table III. *Exps. 1 and 2 (14 days each). Mean balance figures for Ca and P*

Group	Ca	Ca excretion, g.		Ca	P	P excretion, g.		P
	intake	Urine	Faeces	balance	intake	Urine	Faeces	balance
	g.			g.	g.			g.
Exp. 1								
(a)	0.2306	0.0486	0.0041	0.1779	0.2038	0.0129	0.0232	0.1677
(b)	0.2300	0.0691	0.0280	0.1329	0.1879	0.0040	0.0373	0.1466
(c)	0.2321	0.0315	0.0031	0.1976	0.2415	0.0351	0.0253	0.1811
(d)	0.2311	0.0369	0.0225	0.1717	0.2251	0.0240	0.0371	0.1640
Exp. 2								
(a)	0.3934	0.0103	0.0145	0.3686	0.3420	0.0676	0.0302	0.2442
(b)	0.3906	0.0085	0.0380	0.3441	0.3129	0.0297	0.0550	0.2282
(c) (i)*	0.4317	0.0085	0.0170	0.4062	0.4468	0.1536	0.0356	0.2576
(c) (ii)*	0.4207	—	—	—	0.4352	—	—	—
(d) (i)*	0.4279	0.0052	0.0478	0.3749	0.4136	0.1119	0.0607	0.2410
(d) (ii)*	0.4169	—	—	—	0.4033	—	—	—

* Cf. footnotes, Table II.

minerals were derived from Ca phosphate (group (b)) the results were similar but the retentions (especially of Ca) were lower and more Ca was excreted in the faeces, reducing the urinary excretion to 71 % of the total. It should be remembered that the two groups (a) and (b) were not strictly comparable owing to the lower P intake of the latter group. Moreover, it is quite obvious from further experiments (pp. 176 and 184) that at low levels of intake the retention of Ca is markedly affected by the quantity of P in the diet. Further, milk and diet 75 differed with regard to buffering power and acid-base balance. These remarks apply equally to groups (c) and (d) and to all four groups in Exp. 2.

The addition of Na acid phosphate to the milk diet (group (c)) brought the P intake to 90 % of the normal retention and increased the retention of Ca to 85 % by considerably lowering the urinary excretion. The absolute retention of P also improved, though more was lost in the urine. The additional P had a similar effect on the rats receiving the Ca phosphate diet but the retentions of Ca (and of P), though increased, were still inferior to the retentions on the milk diet.

Table IV. *Exps. 1 and 2 (14 days each). Various data for Ca and P retentions and excretions*

Group	% Ca retention	Ca				% P retention	P			
		% Ca intake derived from milk or diet 75	intake mg./g. gain in wt.	% of normal retention at same rate of intake*	% total Ca excretion in the urine		% P intake derived from milk or diet 75	intake mg./g. gain in wt.	% of normal retention at same rate of intake†	% total P excretion in the urine
Exp. 1										
(a)	77.14	89.7	5.74	72.1	92.31	82.30	86.5	5.07	78.4	35.83
(b)	57.77	89.6	5.72	71.9	71.12	78.01	85.6	4.67	71.1	9.74
(c)	85.12	88.7	5.56	70.9	91.09	74.97	73.4	5.79	89.3	58.17
(d)	74.28	89.4	5.78	72.2	62.14	72.85	71.5	5.63	86.5	39.32
Exp. 2										
(a)	93.70	93.9	11.63	112.4	41.73	71.42	91.9	10.12	148.6	69.10
(b)	88.08	93.9	11.60	111.5	18.31	72.93	91.1	9.28	142.2	35.03
(c) (i)‡	94.09	94.8	14.53	139.3	33.40	57.67	78.0	15.04	223.4	81.18
(c) (ii)‡	—	—	11.91	116.9	—	—	—	12.33	185.2	—
(d) (i)‡	87.61	94.7	12.40	122.3	9.81	58.27	76.3	11.99	179.8	64.81
(d) (ii)‡	—	—	10.22	104.0	—	—	—	9.88	152.2	—

* Calculated from data of Sherman & Macleod [1925].

† Calculated from data of Sherman & Quinn [1926].

‡ Cf. footnotes, Table II.

(b) *Exp. 2.* In Exp. 2 it was aimed to supply to groups (a) and (b) quantities of Ca and P just above the normal retentions. Table IV shows that this was satisfactorily achieved for Ca in that the amounts fed surpassed the normal retention figures by about 12%, but that for P the margin was over 40%. This is explained by differences in the shape of the curves relating Ca and P retentions to the weight of the rat [Sherman & Macleod, 1925; Sherman & Quinn, 1926]. In groups (c) and (d) the Ca intakes were relatively higher (some 40 and 20% respectively above the normal) owing to the poor growth of one rat in each group. One rat in group (c) refused food during the last week and the intake of his litter-mate in group (d) had to be cut down accordingly. The marked discrepancy in the P intakes of these groups expressed as % normal retention (Table IV) was due to smaller gain in weight and to higher absolute P intake of one group. When the two unsatisfactory rats are omitted the average Ca and P intakes for the groups drop down to 117 and 185% and 104 and 152% respectively of the normal retentions.

The Ca intake in Exp. 2 was almost twice that in Exp. 1 (Table III), yet, in the presence of a relative surplus of P, the total excretion of Ca decreased because of a marked reduction in the urinary loss, so that more than twice as much Ca was retained and the efficiency of utilization rose to 94% for milk and 88% for Ca phosphate (Table IV). Further addition of P in groups (c) and (d) did not increase the % retention. There was a slight increase in the faecal loss of Ca in groups (a) and (b) in this experiment as compared with groups (c) and (d) of Exp. 1, but it should be pointed out that the shift in the % excretion of Ca from urine to faeces was chiefly due to the decrease of the urinary output.

It is probable that the retention of 94% of milk Ca was not maximal and that it might have been higher if Ca had been given at a level slightly below instead of above that of the calculated normal retentions. The increase in faecal Ca (Table III) supports this view as it indicates that excess unabsorbed Ca was passing through the gut.

The results of the two preliminary experiments throw light on some of the peculiarities noticed in our earlier work [Henry & Kon, 1937, 1]. They do not explain why P was not quantitatively retained, but the relatively low retention of Ca must have been due to an actual shortage of P as it was greatly improved by an increase in the level of P. The Ca of milk appears to be almost completely digestible and to be taken up in the blood stream. In the absence of sufficient P a part of it cannot be retained and is lost to the body through the urine. Hence much more Ca is excreted under these conditions through the kidney than through the gut. The Ca from an inorganic source behaves similarly but appears to be less digestible, since in all comparisons more was found in the faeces. Even then, under certain conditions, over 70 % of it was excreted in the urine. On the Ca phosphate diets more P was also excreted in the faeces and when excretions on these diets are compared with excretions on the milk diets it is found that the excess faecal Ca and P are in the ratios of 1.70 : 1, 1.64 : 1, 0.95 : 1 and 1.23 : 1 respectively for the several milk-Ca phosphate comparisons (Table III), indicating the passage of unchanged CaHPO_4 through the gut. As already stated (p. 175) the milk and Ca phosphate diets were not strictly comparable. The problem of availability of Ca and P from different sources and the question of the metabolism of P were therefore further studied in the following experiments.

EXPERIMENT 3¹ (MAIN EXPERIMENT)

1. *The diets.* Two alterations were made in the composition of the diets. In the first instance liquid raw milk fed separately was replaced by spray-dried milk incorporated in the ration. This greatly facilitated the equalization of mineral intakes between milk and Ca phosphate diets which could be arranged beforehand with certainty. In addition it yielded information on the effect of drying on the availability of milk minerals. The second alteration was in the make-up of the substitute diet carrying the inorganic Ca phosphate. An attempt was made to render it equivalent to milk with respect not only to calorie, Ca and P contents but also to the relative proportions of constituents and to acid-base balance. A new diet was added to study the effect on the retention of P of excess Ca and of subnormal P intakes.

(a) *The basal diets.* The following three basal diets were used: (i) A diet low in both Ca and P, identical with diet 64 of the preliminary experiments (p. 173). (ii) A diet low in Ca but relatively high in P. It was made from diet 64 by adding to it sufficient Na_2HPO_4 , $12\text{H}_2\text{O}$ to raise the P content of the diet to 0.13–0.15 %. This level was calculated from the preliminary experiments (p. 176) and from our previous work [Henry & Kon, 1937, 1] to supply, together with the milk or substitute diet, an excess of P over the normal retentions. (iii) A diet relatively high in Ca but low in P. This was obtained by adding CaCO_3 to the low Ca and P diet to bring the Ca content to 0.20–0.21 %. Table V gives the analyses of these basal diets.

Table V. *Exp. 3. Ca, P and moisture contents of the basal diets*

Basal diet no.	% Ca	% P	% moisture
(i)	0.0195	0.0239	10.43
(ii)	0.0200	0.1489	10.91
(iii)	0.2170	0.0239	10.36

To each of these basal diets suboptimal amounts of Ca and P were added either from milk or from $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$. This was done by mixing with the diets requisite quantities of spray-dried milk or of the substitute diet.

¹ Read in part before the Biochemical Society, 8 October 1938 [Henry & Kon, 1938].

(b) *The substitute diet.* The diet was planned to be equicaloric with the spray-dried milk, to have a similar acid-base balance and to resemble it regarding the protein, fat and lactose contents. As the Ca and P were to be given in inorganic combination as Ca phosphate, casein and lactalbumin were replaced by egg-white. Similarly the salts had to be altered. We are indebted to Dr W. L. Davies for advice on this matter. Information on the salt combinations in milk was taken from his book [Davies, 1936]. The composition of the diet is given in Table VI. It was prepared without the addition of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ and analysed for Ca and P. The calculated amount of Ca phosphate was blended directly with the basal diet and the rest of the substitute diet was then mixed in. For this reason Ca and P figures are not given in Table VI but Table VIII shows that the quantities of these elements in the substitute diet and in milk were practically identical.

(c) *The spray-dried milk.* The sample was dried by Messrs Aplin and Barrett at their Frome Factory in a Kestner plant after preheating. It was stored in sealed tins under N_2 and CO_2 and was 3 months old when used. The composition of the milk is given in Table VI.

Table VI. *Exp. 3. Composition of the substitute diet and of spray-dried milk*

	Substitute diet (as mixed) %	Dried milk %
Butter-fat	28.73	28.97
Egg white	27.63	—
Protein ($\text{N} \times 6.38$)	—	25.94
Lactose	36.57	36.00
NaCl	0.402	—
KCl	0.342	—
K citrate	1.948	—
Mg citrate	0.689	—
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$	3.682	—
Ca	—	0.9454
P	—	0.7307
Moisture	2.67	1.1

(d) *The final diets.* These were mixed in the proportion of 100 g. dried milk or 101.4 g. substitute diet to 569.56 g. of each basal diet. The slight difference

Table VII. *Exp. 3. Composition of the final diets*

Basal diets	...	...	(i) Low Ca and low P		(ii) Low Ca and high P		(iii) High Ca and low P	
			Milk	" CaHPO_4 "	Milk	" CaHPO_4 "	Milk	" CaHPO_4 "
Supplements	...	...	89	90	91	92	93	94
Final diet no.	...	...	89	90	91	92	93	94
Components:								
Egg white*			12.12	16.27	11.94	16.09	12.06	16.21
Butter fat			4.25	8.59	4.19	8.53	4.23	8.57
Maize starch			58.18	58.05	57.32	57.19	57.90	57.76
Castor sugar			8.08	8.06	7.96	7.94	8.04	8.02
Lactose			—	5.53	—	5.53	—	5.53
Ca- and P-free salt mixture†			2.424	2.419	2.389	2.383	2.412	2.407
NaCl			—	0.061	—	0.061	—	0.061
KCl			—	0.052	—	0.052	—	0.052
K citrate			—	0.295	—	0.295	—	0.295
Mg citrate			—	0.104	—	0.104	—	0.104
$\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$			—	—	1.260	1.257	—	—
CaCO_3			—	—	—	—	0.423	0.422
Spray dried whole milk			14.94	—	14.94	—	14.94	—
$\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$			—	0.557	—	0.557	—	0.557

* Dried, heat-coagulated from new laid eggs.

† Henry & Kon [1937, 1].

in quantities between milk and substitute diets was due to difference in moisture content. The ratios in which the diets were mixed were calculated from the relative intakes of milk and basal diet observed in our previous experiments [cf. Table II and Henry & Kon, 1937, 1].

Table VII gives the composition and Table VIII the analyses of the final diets.

Table VIII. *Exp. 3. Analyses of experimental diets*

Diet no.	% Ca	% P	% moisture	Ca : P	% Ca derived from milk or Ca phosphate	% P derived from milk or Ca phosphate	% of normal retention supplied*	
					Ca	P		
89	0.1610	0.1284	9.36	1.25	89.5	84.3	65.1	68.5
90	0.1610	0.1264	9.41	1.27	89.5	84.3		
91	0.1600	0.2367	9.95	0.68	89.3	46.3	63.8	125.5
92	0.1607	0.2328	10.01	0.68	89.3	46.3		
93	0.3284	0.1283	9.36	2.56	43.3	84.3	154.5	74.7
94	0.3280	0.1271	9.39	2.58	43.5	84.3		

* These figures were calculated after the experiment was completed from data of Sherman & Macleod [1925] and Sherman & Quinn [1926].

2. *The animals.* Six litters were used, each consisting of 6 male rats 23–24 days old and weighing 42–56 g. The animals were distributed by lot but in cases where differences in weight between litter-mates were appreciable uniform pairs were first selected for the milk-Ca phosphate comparisons and the final allocation was by toss of coin. The animals were numbered consecutively, rats 1–6, 7–12 etc. being litter-mates. The two types of metabolic cages used and the methods of feeding, giving vitamin additions, collecting excreta etc. were as already described [Henry & Kon, 1937, 1]. Distilled water was given for drinking. The experimental diets were fed for 4 days before the collection of excreta was started. The experiment lasted 5 weeks, the cages being washed once a week. At the end of the experiment the rats were killed by coal gas and the carcasses, after removal of the intestinal contents, were ashed and analysed for Ca and P. In ashing, 20 ml. of a 10% solution of Na acetate were added to supply fixed base, as without this precaution some loss of P occurred in our earlier experiments [Henry & Kon, 1937, 1].

3. *Results.* The results of this experiment are set out in Tables IX–XIII. Table IX gives the individual and mean data for the weights of rats and faeces; Tables X and XI give various data for individual rats concerning the intakes, excretions and retentions of Ca and of P. Table XII gives group means of data calculated from the two previous tables. Table XIII contains the results of statistical tests of significance of some of the differences. For this purpose the paired *t*-test of "Student" [1908; 1925] was used.

Towards the end of the experiment rat no. 34 on diet 92 showed a definite loss of appetite and passed blood in the faeces. During the last few days, therefore, no attempt was made to equalize the food intakes of this rat and of its litter-mate, no. 33, on diet 91. When the animals were killed for analysis it was found that the intestinal contents of rat 34 were, owing to the presence of blood, of a chocolate colour instead of the normal black. This was most probably due to internal injury caused by the animal nibbling off part of a metal tag on the cage. As can be seen from Table X the faeces of this animal contained abnormally large quantities of Ca and P, doubtless due to the haemorrhage. The figures for this rat (and for its litter-mates when necessary for comparative purposes) have been omitted from certain means.

Table IX. *Exp. 3 (35 days). Individual and mean data for the weights of rats and of faeces*

Rat no.	Wt. of rat, g.		Wt. of faeces* g.	Rat no.	Wt. of rat, g.		Wt. of faeces* g.
	Initial	Final			Initial	Final	
Diet 89				Diet 90			
1	62	180	6.27	2	60	174	8.12
7	56	135	4.32	8	56	131	5.29
13	53	156	5.65	14	55	158	6.07
19	64	148	5.15	20	63	148	5.23
25	55	138	4.57	26	50	140	4.70
31	56	168	6.22	32	55	162	5.69
Mean (i)†	57.7	154.2	5.36		56.5	152.2	5.85
Mean (ii)‡	—	—	—		56.8	150.2	5.88
Diet 91				Diet 92			
3	63	166	5.22	4	62	161	6.12
9	62	148	4.65	10	62	149	5.20
15	60	155	5.37	16	60	146	5.38
21	63	149	4.82	22	63	153	5.12
27	60	167	5.00	28	57	164	5.58
33	59	137	4.68	34	57	124	8.25
Mean (i)†	61.2	153.7	4.96		—	—	—
Mean (ii)‡	61.6	157.0	5.01		60.8	154.6	5.48
Diet 93				Diet 94			
5	64	159	5.53	6	61	148	6.68
11	55	129	5.90	12	55	125	6.23
17	53	127	4.60	18	54	124	4.68
23	59	142	4.92	24	58	138	5.82
29	51	130	5.06	30	50	136	5.41
35	51	115	6.32	36	55	120	5.76
Mean (i)†	55.5	133.7	5.39		55.5	131.8	5.76
Mean (ii)‡	—	—	—		55.6	134.2	5.76

* Air-dry.

† Mean (i) = Mean for 6 animals.

‡ Mean (ii) = Mean for 5 animals, omitting litter VI (rats 31–36 incl.).

Table X. *Exp. 3 (35 days). Individual figures for the intakes and excretions of Ca and P*

Rat no.	Diet intake g.	Ca intake g.	Ca excretion, g.		P intake g.	P excretion, g.	
			Urine	Faeces		Urine	Faeces
			Diet 89				
1	377.93	0.6085	0.1293	0.0038	0.4853	0.0069	0.0577
7	286.88	0.4619	0.0763	0.0045	0.3684	0.0120	0.0341
13	359.96	0.5795	0.0901	0.0049	0.4622	0.0113	0.0459
19	332.99	0.5361	0.0888	0.0072	0.4276	0.0236	0.0462
25	306.27	0.4931	0.0795	0.0048	0.3933	0.0118	0.0399
31	386.46	0.6222	0.1175	0.0031	0.4962	0.0142	0.0534
Diet 90							
2	377.70	0.6081	0.1541	0.0181	0.4774	0.0046	0.0842
8	288.00	0.4637	0.1008	0.0100	0.3640	0.0090	0.0528
14	359.59	0.5789	0.1068	0.0195	0.4545	0.0081	0.0582
20	331.17	0.5332	0.0860	0.0167	0.4186	0.0099	0.0562
26	306.07	0.4928	0.0946	0.0249	0.3869	0.0039	0.0498
32	385.33	0.6204	0.0971	0.0277	0.4871	0.0136	0.0553

Table X (cont.)

Rat no.	Diet intake g.	Ca intake g.	Ca excretion, g.		P intake g.	P excretion, g.	
			Urine	Faeces		Urine	Faeces
			Diet 91				
3	333.33	0.5333	0.0101	0.0027	0.7890	0.3039	0.0501
9	314.50	0.5032	0.0089	0.0027	0.7444	0.3029	0.0483
15	328.90	0.5262	0.0020	0.0038	0.7785	0.3065	0.0530
21	328.43	0.5255	0.0043	0.0037	0.7774	0.3299	0.0493
27	365.83	0.5853	0.0085	0.0036	0.8659	0.3673	0.0489
33	313.92	0.5023	0.0066	0.0041	0.7430	0.3329	0.0499
Diet 92							
4	334.48	0.5375	0.0077	0.0116	0.7787	0.2991	0.0692
10	314.10	0.5048	0.0021	0.0119	0.7312	0.3018	0.0577
16	328.33	0.5276	0.0017	0.0163	0.7644	0.3189	0.0636
22	328.11	0.5273	0.0060	0.0174	0.7638	0.3142	0.0633
28	365.75	0.5878	0.0076	0.0116	0.8515	0.3443	0.0628
34	297.62	0.4783	0.0102	0.2082	0.6929	0.2543	0.1171
Diet 93							
5	337.19	1.1073	0.4784	0.0993	0.4326	0.0048	0.0350
11	290.41	0.9537	0.2964	0.1931	0.3726	0.0045	0.0370
17	292.77	0.9615	0.2993	0.1646	0.3756	0.0052	0.0322
23	323.52	1.0624	0.3896	0.1222	0.4151	0.0063	0.0385
29	288.87	0.9486	0.3506	0.1235	0.3706	0.0056	0.0417
35	277.78	0.9122	0.2856	0.1852	0.3564	0.0056	0.0543
Diet 94							
6	336.08	1.1023	0.4344	0.2035	0.4272	0.0062	0.0774
12	290.12	0.9516	0.3328	0.2330	0.3687	0.0054	0.0836
18	292.13	0.9582	0.3181	0.1800	0.3713	0.0067	0.0574
24	322.33	1.0572	0.3297	0.2475	0.4097	0.0056	0.0720
30	288.00	0.9446	0.3721	0.1942	0.3660	0.0044	0.0683
36	276.80	0.9079	0.4429	0.0956	0.3518	0.0038	0.0572

Table XI. *Exp. 3 (35 days). Individual figures for the Ca and P contents of the rat carcasses*

Rat no.	Wt. of carcass g.	Ca in carcass g.	Esti- mated de- posited	P in carcass g.	Esti- mated de- posited	Rat no.	Wt. of carcass g.	Ca in carcass g.	Esti- mated de- posited	P in carcass g.	Esti- mated de- posited
			Ca, g.*		P, g.*				Ca, g.*		P, g.*
Diet 89						Diet 90					
1	178	0.8346	0.4752	0.7243	0.4203	2	171	0.7624	0.4146	0.6764	0.3822
7	133	0.7471	0.4107	0.6151	0.3464	8	129	0.7084	0.3720	0.5972	0.3285
13	154	0.7572	0.4762	0.6504	0.4102	14	153	0.7591	0.4675	0.6453	0.3960
19	145	0.8297	0.4236	0.6653	0.3477	20	147	0.8128	0.4130	0.6590	0.3464
25	133	0.7377	0.4262	0.6082	0.3455	26	134	0.6935	0.4103	0.5790	0.3401
31	165	0.8808	0.5067	0.7337	0.4567	32	157	0.8410	0.4735	0.6922	0.4201
Diet 91						Diet 92					
3	162	0.8840	—	0.7360	—	4	157	0.8793	—	0.7221	—
9	144	0.8685	—	0.6784	—	10	145	0.8588	—	0.6814	—
15	151	0.8412	—	0.6844	—	16	142	0.8251	—	0.6605	—
21	147	0.9165	—	0.7098	—	22	149	0.9045	—	0.6999	—
27	162	0.9161	—	0.7302	—	28	158	0.8914	—	0.7167	—
33	131	0.8858	—	0.6521	—	34	120	0.7909	—	0.5948	—
Diet 93						Diet 94					
5	158	0.8765	0.5055	0.6868	0.3730	6	144	0.7883	0.4347	0.6247	0.3256
11	123	0.8084	0.4780	0.6112	0.3473	12	120	0.7047	0.3743	0.5500	0.2861
17	120	0.8015	0.5205	0.6000	0.3598	18	120	0.7494	0.4631	0.5693	0.3245
23	138	0.9123	0.5379	0.6724	0.3796	24	133	0.8350	0.4669	0.6345	0.3467
29	125	0.7757	0.4869	0.5790	0.3354	30	130	0.6492	0.3660	0.5458	0.3069
35	112	0.8146	0.4739	0.5845	0.3322	36	116	0.7558	0.3883	0.5711	0.2990

* Cf. p. 183.

Table XII. *Exp. 3 (35 days)*(a) *Group means calculated from data in Table X*

Diet no.	Diet intake g.	Ca excretion, g.		Ca balance g.	% Ca retention	% total Ca excretion in urine	P intake g.	P excretion, g.		P balance g.	% P retention	% total P excretion in urine
		Urine	Faeces					Urine	Faeces			
89 (i)*	341.75	0.5502	0.0047	0.4486	81.65	95.11	0.4388	0.0132	0.0462	0.3793	86.45	22.35
90 (i)*	341.31	0.5495	0.1066	0.4235	77.06	84.29	0.4314	0.0082	0.0594	0.3638	84.34	12.32
90 (ii)*	332.51	0.5353	0.1085	0.4090	76.49	85.58	0.4203	0.0071	0.0602	0.3529	84.03	10.84
91 (ii)*	334.20	0.5347	0.0068	0.0033	98.12	62.82	0.7910	0.3221	0.0499	0.4190	52.98	86.52
92 (ii)*	334.16	0.5370	0.0050	0.0138	96.50	25.91	0.7779	0.3157	0.0633	0.3989	51.25	83.27
91 (i)*	330.82	0.5293	0.0067	0.0035	98.08	62.63	0.7830	0.3239	0.0499	0.4092	52.23	86.59
93 (i)*	301.76	0.9910	0.3500	0.1480	49.75	69.77	0.3871	0.0053	0.0398	0.3420	88.22	12.01
94 (i)*	300.91	0.9870	0.3717	0.1923	42.81	65.98	0.3825	0.0053	0.0693	0.3078	80.48	7.24
94 (ii)*	305.93	1.0028	0.3574	0.4337	43.23	62.72	0.3886	0.0057	0.0717	0.3112	80.05	7.44

(b) *Group means calculated from data in Table XI*

Diet no.	Wt. of carcass g.	Ca in carcass		Estimated† initial Ca, g.	Ca deposited g.	P in carcass		Estimated† initial P, g.	P deposited g.	Ca : P ratio of carcasses
		g.	%			g.	%			
89 (i)*	151.3	0.7979	0.5305	0.3448	0.4531	0.6662	0.4421	0.2784	0.3878	1.197
90 (i)*	148.5	0.7629	0.5162	0.3377	0.4252	0.6415	0.4336	0.2726	0.3689	1.190
90 (ii)*	146.8	0.7472	0.5123	0.3317	0.4155	0.6314	0.4321	0.2728	0.3586	1.186
91 (ii)*	153.2	0.8853	0.5790	0.3606	—	0.7078	0.4624	0.2888	—	1.250
92 (ii)*	150.2	0.8718	0.5809	0.3536	—	0.6961	0.4636	0.2972	—	1.252
91 (i)*	149.5	0.8854	0.5952	0.3662	—	0.6985	0.4683	0.2893	—	1.268
93 (i)*	129.3	0.8315	0.6481	0.3311	0.5005	0.6223	0.4340	0.2678	0.3545	1.338
94 (i)*	127.2	0.7471	0.5897	0.3315	0.4156	0.5826	0.4593	0.2678	0.3148	1.282
94 (ii)*	129.4	0.7453	0.5773	0.3243	0.4210	0.5849	0.4527	0.2669	0.3180	1.274

* Cf. footnote to Table IX.

† Cf. p. 183.

Table XIII. *Exp. 3. Statistical treatment of some of the differences*

Dietary difference tested*	Calcium			Phosphorus		
	Difference	Standard error of the mean	P†	Difference	Standard error of the mean	P†
	(a) % retention at different levels of Ca and P intake					
91-89	+16.44	±0.84	1 : over 11,000, signif.	—	—	—
92-90	+20.01	±1.64	1 : 3570, signif.	—	—	—
93-89	—	—	—	+1.77	±1.25	1 : 5, not signif.
94-90	—	—	—	-3.86	±0.93	1 : 111, signif.
	(b) % retention from milk or CaHPO ₄					
89-90	+4.58	±1.15	1 : 96, signif.	+2.11	±0.95	1 : 13, not signif.
91-92	+1.62	±0.44	1 : 47, signif.	+1.73	±0.72	1 : 14, not signif.
93-94	+6.94	±0.88	1 : 1850, signif.	+7.74	±1.71	1 : 161, signif.
	(c) Faecal excretion from milk or CaHPO ₄					
90-89	+0.0148	±0.0028	1 : 312, signif.	+0.0132	±0.0034	1 : 86, signif.
92-91	+0.0105	±0.0011	1 : 1430, signif.	+0.0134	±0.0017	1 : 735, signif.
94-93	+0.0443	±0.0314	1 : 5, not signif.	+0.0295	±0.0063	1 : 184, signif.
	(d) Urinary excretion from milk or CaHPO ₄					
90-89	+0.0097	±0.0073	1 : 4, not signif.	-0.0050	±0.0020	1 : 18, not signif.
92-91	-0.0018	±0.0014	1 : 4, not signif.	-0.0064	±0.0061	1 : 3, not signif.
94-93	+0.0217	±0.0314	1 : 2, not signif.	0.0000	±0.0006	Not signif.

* The figures in this column refer to the numbers of the diets.

† P=probability that a mean difference at least as great as the observed mean difference would have arisen by random sampling from a homogeneous population.

The two diets containing additional Ca, nos. 93 and 94, appeared to be less palatable than the other diets. Less was eaten, there was a good deal of scattering and the animals grew less well on these diets (Tables IX, X and XIIa). Though great care was taken in collecting the scattered diet as soon as possible, some contamination with urine could not be avoided. Such diet was then carefully dried by pressing between filter papers. These were then extracted with dilute HNO₃ and the washings were added to the urines. That this procedure was satisfactory and that the analytical results for groups 93 and 94 were correct can be shown in the following way. The animals receiving diets 91 and 92 did not scatter the food at all. It was therefore possible to calculate their initial stores of Ca and P from the analyses of their carcasses at the end of the experiment and from their Ca and P balances. These figures were then used to estimate [Henry & Kon, 1937, 1] the initial mineral stores of their litter-mates in groups 89 and 90, and 93 and 94. From these data and the carcass analyses the Ca and P depositions in these rats were calculated. Table XII a and b shows that the agreement between figures obtained in this way and the Ca and P balances of these rats is very good, especially as no litter-mates were actually analysed at the beginning of the experiment. These results justify confidence in the use of the urinary analyses for groups 93 and 94.

Turning now to a discussion of the results it is convenient to review them under two headings. The effects of changing the levels of Ca and P will be considered first and the differences in availability of Ca and P according to source will be dealt with later.

(a) *Effect of level of Ca and P in the diet.* When Ca and P were both supplied in quantities well below the normal retentions (diets 89 and 90) the results (Tables X and XIIa) were very similar to those obtained in the previous experiments in this paper (p. 174) and in earlier work [Henry & Kon, 1937, 1].

Again the retention of Ca was about 80 %, and some 90 % of the excreted Ca was found in the urine. The retention of P, amounting to some 85 %, was also not quantitative. In these respects it mattered little whether the minerals were derived from milk or from an inorganic source, there was no qualitative difference in their behaviour. It should be noted that there was a closer agreement between the milk and non-milk diets than in the preliminary experiments (p. 175), most probably because the two types of diets were this time more strictly comparable.

The most dramatic change followed the increase in dietary P (Tables X and XIIa). This brought about a very marked increase in the retention of Ca, with the result that the % retention rose to 98.1 and 96.5 respectively for the two types of diets. Table XIIIa shows the high statistical significance of this change. Our supposition that the originally low retention of Ca was due to a shortage of P was thus definitely confirmed. The excretion of Ca per g. Ca intake decreased almost tenfold. This was almost entirely due to a diminished excretion of Ca in the urine, the fall amounting to some 160–180 mg. per g. Ca intake or about 93–95 % of the original urinary Ca. The faecal Ca also fell by some 30 and 27 % respectively, but this amounted to only 3–10 mg. per g. Ca intake, as can be calculated from data given in Tables X and XIIa. As a result a higher proportion of the small quantity of Ca still lost on these diets was found in the faeces; this is specially noticeable on the Ca phosphate diet. The increased retention of Ca was obviously accompanied by increased deposition of P, 567 mg. and 488 mg. of P respectively being deposited per g. increase in Ca deposition.

The increase in dietary Ca, the P being left unchanged (diets 93 and 94), had a much smaller effect on the metabolism of P. The % retention increased slightly but not significantly for the milk diet (Tables X, XIIa and XIIIa) but dropped significantly, though not to a marked extent, for the Ca phosphate diet. This latter effect was due to increased faecal loss (Tables X and XIIa). In both cases the urinary excretion was diminished.

(b) *The availability of Ca and P from milk and from calcium phosphate.* In all three comparisons the rats retained more Ca and more P from milk than from the inorganic source (Tables X and XIIa). The differences are more pronounced for Ca and Table XIIIb shows that in every case the superiority of milk is statistically significant.¹ Under conditions leading to maximal retention of Ca (diets 91 and 92) the Ca from Ca phosphate is retained very efficiently, all but 3.5 % being deposited in the body. This is only 1.6 % less than the retention from milk and it is interesting that such a small difference should be statistically significant. This is probably because of the internal consistency of the results yielded by the two groups of rats, which can be calculated from Table X.

Of the differences in P retention only one, that between diets 93 and 94, is statistically significant; the others, though suggestive with odds of 13:1 and 14:1 against the results being due to chance, are not. The differences in availability of minerals between the milk and non-milk diets are mostly due to higher faecal excretions of both Ca and P on the Ca phosphate diet. These differences in faecal excretions are all statistically significant (Table XIIIc) with the exception of the comparison of diets 93 and 94 in respect of Ca. The urinary excretions differ less and not always in the same direction. They are greater for

¹ Strictly speaking only 90 % of the Ca was derived from milk or Ca phosphate. The remaining 10 % from the basal diet was common to both treatments. A correction to this effect might be introduced in the calculations as has been done by Ellis & Mitchell [1933]. For reasons already given [Henry & Kon, 1937, 1] we prefer not to do this.

milk rats in one case for Ca (diets 91 and 92) and in two cases for P. Table XIII*d* shows that these differences are not significant. The faecal loss of both minerals is invariably higher on the Ca phosphate diet and the Ca : P ratio of the excess excretions suggests that the poorer availability of the inorganic additions was due to non-absorption of some of the Ca phosphate which passed unchanged through the gut. The ratios are 1.12 : 1, 0.78 : 1 and 1.50 : 1 respectively for the three comparisons. The Ca : P ratio of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ is 1.29 : 1.

DISCUSSION

1. *The metabolism of Ca and P*

Most of the Ca excreted by adult animals on normal diets is found in the faeces and until recently it was commonly believed that the gut functions as an excretory organ for this element. It was thought that Ca was absorbed by the small intestine and re-excreted in the lower bowel. A summary of relevant work will be found in the reviews of Stewart & Percival [1928] and of Hunter [1930]. More recently, however, Nicolaysen [1934] and Christiansen [1936] came to the conclusion that much of the evidence brought forward in favour of an active excretion of Ca by the gut was either experimentally unsatisfactory or inconclusive. Their own experiments on dogs [Nicolaysen, 1934] or rats [Nicolaysen, 1937], and on rabbits and goats [Christiansen, 1936], did not support the view that the lower bowel excretes Ca. Recently also the careful observations of Welch *et al.* [1936] and of Johnson [1937] have shown that in man only negligible quantities of Ca are excreted by the colon. Wright *et al.* [1938] have come to a similar conclusion regarding the cat. On the other hand Cowell [1937] has produced experimental arguments for the excretion of Ca into the large intestine of the rabbit which cannot be lightly dismissed and Dr Quinn of the Onderstepoort Laboratory, who is studying the digestive processes of sheep by means of fistulae at several intestinal levels, is of the opinion that the possibility of active excretion of Ca in the colon of ruminants must be seriously considered [private communication]. It is quite possible that herbivora behave differently in this respect from omnivorous or carnivorous animals. For both the latter the recent evidence speaks strongly against the active participation of the colon in Ca metabolism and in our opinion our work on young growing rats lends definite support to this view. In the first instance we find that on diets supplying a sufficiency of P but not enough Ca for proper calcification, the latter mineral is most efficiently retained, the daily loss in the period of rapid growth amounting to only 0.2 mg. daily in the urine and 0.1 mg. in the faeces (Table XII*a*, diet 91). Under such conditions there can be no doubt that there is no significant excretion of Ca through the colon. But even when the supply of P is so reduced that the food Ca, though limited in amount, cannot be efficiently deposited in the bone and some of it must be excreted, this excretion takes place almost exclusively through the urinary path. The daily loss amounts in this case to 2.8 mg. in the urine but still to only 0.1 mg. in the faeces (Table XII*a*, diet 89). Admittedly these very low faecal excretions are found only on the diets in which milk supplies most of the Ca. When this is derived from Ca phosphate the daily losses of Ca in the faeces rise to 0.4 mg. (diet 92, Table XII*a*) and 0.6 mg. (diet 90, Table XII*a*) respectively, but the faecal P is correspondingly increased and, in all probability, the higher faecal Ca on these diets does not indicate any active excretion of Ca through the large intestine but only that a part of the Ca phosphate has passed through the gut unchanged. In this case the difference in the urinary loss of Ca is even more spectacular, 0.14 mg. daily for the diet

containing adequate P (diet 92, Table XIIa) and more than 20 times this amount, 3.0 mg., daily for the low-P diet (diet 90, Table XIIa).

The efficiency with which growing rats are able, under conditions favouring the uptake of Ca, to retain this element has already been pointed out by Ellis & Mitchell [1933]. These authors expressed the view that "in the growing rat and possibly in all growing animals there is no integral requirement of Ca for maintenance", and in the two preliminary reports on our present work [Henry & Kon, 1937, 2; 1938] we fully endorsed this view. Indeed, the retention by young rats of over 98 % of the ingested Ca, not in a short metabolic period but in the course of 5 weeks during which they almost trebled their initial weights, can hardly be otherwise interpreted. For reasons more fully set out below we believe that it would be difficult to improve on the retentions observed in our present studies. Strictly speaking, therefore, the daily loss of some 0.3 mg. Ca may represent the unavoidable minimum. Two-thirds of it are due to urinary leakage of circulating Ca and the rest probably represents the Ca of digestive secretions which have not been reabsorbed, and the Ca of bacterial bodies. According to Sherman & Macleod [1925] the intestinal tract of the rat contains only negligible quantities of Ca and therefore the contribution of the shed epithelium is of no consequence in this respect.

We have just shown (p. 184) that under certain conditions the loss of Ca was primarily due to a relative shortage of P. The cause of the P loss is, however, not so easily explained. It is clear that it could not be due in the first instance to a shortage of Ca and addition of Ca to the diet did not improve the retention. It appears, therefore, that even under optimal conditions on the milk diets a definitely greater loss of P than of Ca is unavoidable. The difference lies mainly in the magnitude of the faecal excretions: 1.1 mg. daily, or 10.4 % of the intake, for P (Table XIIa, diet 93), as against 0.1 mg. daily, or 0.6 % of the intake, for Ca (Table XIIa, diet 91). The urinary losses on these diets were very similar: 0.15 mg. daily or 1.4 % of intake for P and 0.2 mg. or 1.3 % respectively for Ca. It is possible that a part of the faecal loss represents P that is not available. The diets contained some 57 % of maize starch with a P content of 0.016 % [Henry & Kon, 1937, 1]. If we assume that 50 % of this P is phytin-P—a generous estimate [cf. McCance & Widdowson, 1935]—and that 75 % of it is not available to the rat [Lowe & Steenbock, 1936], we arrive at an estimate of about 12 mg. faecal P due to unabsorbable phytin. This is, however, not enough to explain the difference between the faecal excretions of Ca and P, as at least 30 mg. of faecal P are not allowed for. It may be pointed out here that Brooke & Smith [1933] observed in rats on a ration very deficient in Ca and low in P daily faecal excretions of 0.3 mg. Ca and 1.3 mg. P and that Nicolaysen [1937] found in Ca and P starvation corresponding figures of 0.45 mg. Ca and 1.0 mg. P. Using a diet containing 0.15 % Ca and 0.4 % P (of which 0.21 % was derived from K_2HPO_4) Rottensten [1938] noticed daily faecal excretions of 0.4 mg. Ca and 2.4 mg. P.

When allowance is made for the faecal P which may have been of phytin origin and, in the case of the diets containing $CaHPO_4 \cdot 2H_2O$, for the P from the unabsorbed Ca phosphate (cf. p. 185), there is evidence suggesting that the rest of the faecal P had been absorbed before it found its way into the gut. Thus, with diet 91, 99.4 % of the total Ca was absorbed (retained or excreted in the urine) which would leave practically the whole of the P in assimilable form. It seems therefore very probable that part of the faecal P was assimilated and then passed into the gut in the digestive juices and as shed epithelium. The findings of Johnson [1937] and Welch *et al.* [1936] on man, and Wright *et al.* [1938]

on the cat make it improbable that P is actively excreted in the large intestine. Intestinal epithelium doubtless contains much more P than Ca, but judging from the figures given by Johnson [1937] and Welch *et al.* [1936] for the P content of the material from isolated caeca, it is doubtful whether it makes an important contribution to faecal P. From the available recent analyses of digestive juices (cf. Baxter [1933] for mixed saliva, Gamble & McIver [1928], Ågren [1935], Ball [1930] and Walsh & Ivy [1928] for gastric and pancreatic juices; the latter, Drury [1924], Jones & Laing [1934] and Cheymol & Quinquaud [1937] for bile, and de Beer *et al.* [1935], Herrin [1935] and Wright *et al.* [1938] for *succus entericus*) it appears that they contain more Ca than P. Therefore, the mechanism for reabsorption of Ca must be much more efficient than that for P. It seems very probable that this is because a large part of the P present in the gut becomes fixed by the intestinal bacteria and is thus no longer available to the host. It has been shown by Osborne & Mendel [1914] that on low-residue diets like the diets used in the present study, bacterial bodies account for about 40% of the dry weight of rat faeces. P is a more essential component of bacteria than Ca, and though both vary within very wide limits according to bacterial species and to the composition of the medium, authorities agree that bacterial ash contains more of the former than of the latter [Topley & Wilson, 1936; Buchanan & Fulmer, 1928]. Barber [1931], who analysed many species, gives for various coliform organisms values for P of about 1.5% and for Ca around 0.2% of the dry weight. It would be idle to speculate as to the exact P content of the bacterial flora of rats, nevertheless, the following calculation yields very plausible figures.

Table XIV. *Faecal excretion of P per g. food intake, P intake or per g. faeces*

	Total solid intake g.	P intake g.	Faecal P mg.	Wt. of faeces g.	Faecal P/g. total solid intake		Faecal P/g. P intake		Faecal P/g. faeces	
					mg.	%*	g.	%*	mg.	%*
Raw and pas- teurized milk†	367.89	0.5132	81.25	8.69	0.2209	100	0.1583	100	9.35	100
Exp. 1										
Milk	129.03‡	0.2038	23.20	2.49	0.1798	81	0.1138	72	9.32	100
Milk + Na ₂ HPO ₄	132.12‡	0.2415	25.30	2.62	0.1915	87	0.1048	66	9.66	103
Exp. 2										
Milk	148.19‡	0.3420	30.20	3.80	0.2038	92	0.0833	53	7.95	85
Milk + Na ₂ HPO ₄	146.67‡	0.4468	35.60	3.87	0.2427	110	0.0797	50	9.20	98
Exp. 3										
Diet 89	341.75	0.4388	46.20	5.36	0.1352	61	0.1053	67	8.62	92
Diet 91	334.20	0.7910	49.90	5.01	0.1493	68	0.0631	40	9.96	107
Diet 93	301.76	0.3871	39.80	5.39	0.1319	60	0.1028	65	7.38	79

* Taking the figures for raw and pasteurized milk as 100.

† Henry & Kon [1937, 1].

‡ On the assumption that the milk contained 12.5% total solids.

Table IX shows that rats receiving diet 89 excreted on the average in the course of 5 weeks 5.36 g. of air-dry faeces. The faecal P output was 46.2 mg. (Table XII a) or 8.61 mg. P per g. faeces (Table XIV). On the assumption that 1 g. faeces contained 400 mg. bacteria [Osborne & Mendel, 1914] this works out at 2% P in bacterial bodies. A similar calculation for diet 91 gives 2.5% P and for diet 93, 1.8%; corresponding figures for Ca, omitting diet 93, are 0.22% and 0.16% respectively. The figures both for Ca and P are of the order quoted by Barber [1931] for various bacteria. If allowance were made for possible phytin-P not available to bacteria, the agreement with respect to P would be even better.

It seems therefore that as the faecal Ca on these diets can be entirely accounted for by Ca present in bacterial bodies the loss by this route could hardly be lowered. Moreover, the loss of 1.30 mg. Ca/g. faeces on raw milk diets (cf. Henry & Kon [1937, 1] and Exp. 1 of the present work) was greater than the figure of 0.77 mg. Ca/g. faeces obtained with the spray-dried milk diet in Exp. 3. It would appear therefore that drying, like pasteurization, has no untoward effect on the availability of milk-Ca for rats.

As a further argument in favour of the supposition that faecal P is mainly bacterial P, a comparison of all faecal P excretions on milk diets¹ shows that they agree much better when calculated per g. faeces than per unit of food intake or of P intake (Table XIV). A similar argument in favour of the bacterial origin of the faecal K has already been advanced by Peters [1933].

We are thus led to believe that there is no qualitative difference between the metabolism of Ca and that of P in the young growing rat. Neither Ca nor P can be adequately retained in the absence of sufficient quantities of P or Ca. When the available supply of the complementary element is adequate and that of the other is less than normal, only insignificant amounts of either Ca or P can leak out through the kidney.

The reabsorption of the quantities thrown into the gut with the digestive juices seems also to be equally efficient, the disparity in the amounts eventually lost not being due to any physiological or threshold differences but incidental to the symbiosis of host and intestinal flora. In neither case can we find any evidence of active excretion into the lower gut which, in the rat at any rate, does not in our opinion assume the functions of a vicarious kidney.

In our earlier experiments [Henry & Kon, 1937, 1] we found that when the supply of both Ca and P was inadequate, the requirements of the extra-skeletal structures for P were covered to a greater extent than those of the bones. Similar calculations applied to our present results yielded the values given in Table XV.

Table XV. *Exp. 3. Extent to which the requirements of the bones and extra-skeletal structures for P were satisfied by the rats receiving the experimental diets**

Diet no.	% normal P (and Ca) in bones	% normal P in tissues
89	65.7	85.6
90	64.1	85.1
91	71.7	85.3
92	72.7	84.9
93	85.3	77.8
94	78.6	76.5
Liquid milk†	61.0	82.7

* For method of calculation see Henry & Kon [1937, 1].

† Henry & Kon [1937, 1].

The results with diets 89 and 90, low in Ca and in P, agree very well with our previous findings. The addition of extra P (diets 91 and 92), and to a much greater extent the addition of extra Ca (diets 93 and 94), shift the P saturation from the soft tissues and blood to the bones which, when extra Ca is added,

¹ We have not carried out similar calculations for the diets containing Ca phosphate as they would be complicated by the presence in the gut of the unchanged compound (cf. pp. 177 and 185), especially as it was less well utilized in the preliminary experiments than in the final experiment. This can be easily seen by comparing the faecal excretions on milk and Ca phosphate diets in the three experiments.

appear to take precedence in their affinity for this element. It is possible that the inferior gain in weight of the rats on these diets may have had some effect on the result. It is of great interest, however, that Schneider & Steenbock [1938] have observed a similar effect in the presence of vitamin D on diets containing much more Ca than P.

2. The availability of Ca and P from milk and from inorganic sources

Experiments carried out on dogs some 20 years ago by Mendel and his collaborators [Givens & Mendel, 1917; McClugage & Mendel, 1918] showed that the Ca of milk was better utilized than that of Ca lactate or of CaCO_3 . Previously, Aron & Frese [1908] had reported that the growing dog could obtain its Ca from Ca phosphate as efficiently as from milk. More recently Sherman & Campbell [1935; 1937] and Gaunt *et al.* [1938] showed that improvements in certain diets brought about by addition of milk could to a large extent be reproduced by the addition of CaCO_3 or of Ca lactate and Na_2HPO_4 . Fairbanks & Mitchell [1936] found that the Ca of a diet containing 0.18 % of the element, mostly in the form of dicalcium phosphate, was completely utilized by young growing rats. Stearns & Jeans [1934] found that children retained Ca and P approximately as well from CaHPO_4 and $\text{Ca}_3(\text{PO}_4)_2$ as from milk. Finally Coward *et al.* [1938] came to the conclusion that the Ca of pasteurized milk is no more easily available for the growing rat than the Ca of inorganic salts (Ca phosphate plus Ca lactate). It has already been pointed out by one of us [Kon, 1938] and also by Gaunt *et al.* [1938] that this conclusion was not justified by the available experimental data.

As far as they go these studies appear to indicate that if there is a difference between the availabilities of Ca from milk and from inorganic sources it cannot be very marked and our metabolic experiments confirm such a view. We find that though spray-dried milk is a better source of Ca than CaHPO_4 , the difference between them under conditions of optimal retention is small. It should be pointed out that we used commercial Ca phosphate and that though the analysis of the salt tallied very well with the formula, it is possible that it was chemically not quite pure.

Another point to remember is that the degree of saturation of the body stores of Ca has a marked effect on the avidity with which the mineral is deposited [Fairbanks & Mitchell, 1936; Rottensten, 1938] and it is possible that the gap between milk and Ca phosphate as sources of Ca would be different at other levels of intake.

Ellis & Mitchell [1933] found that pasteurization lowered the availability of milk-Ca. We [Henry & Kon, 1937, 1] have been unable to confirm this finding. It is of interest that the figure obtained by us now for the availability of the Ca of a diet containing dried milk—98 %—is the same as that found by Ellis & Mitchell [1933] for a diet containing raw liquid milk. Since then Fairbanks & Mitchell [1937] have reported that Ca from spray-dried skim milk was some 7 % less available than the Ca from raw liquid skim milk, while Fairbanks & Mitchell [1938] obtained an average retention of Ca of only 85 % on a diet containing this type of dried milk. Already Ellis & Mitchell [1933] had expressed the view that the lowering of the availability of Ca in milk by pasteurization was due to a partial destruction of vitamin C. To this Fairbanks & Mitchell [1937] have added now what appears to be a striking confirmation. In preliminary experiments in which dried milk was fed to rats as a source of Ca, with and without the addition of 1 mg. daily of crystalline vitamin C, they found with a high

degree of probability that the administration of ascorbic acid improved the utilization of Ca. Our present experience is against such a view and the problem is now under investigation in our laboratory.

SUMMARY

1. The balance of Ca and of P derived either from milk or from $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ has been studied in three separate metabolic experiments on young male rats.
2. In all comparisons the Ca of milk was found to be more available than the Ca of $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$.
3. In the presence of extra P (as $\text{Na}_2\text{HPO}_4 \cdot 12\text{H}_2\text{O}$) sub-optimal quantities of Ca were retained from spray-dried milk with an efficiency of 98.1 % and from $\text{CaHPO}_4 \cdot 2\text{H}_2\text{O}$ with an efficiency of 96.5 %. The difference between these two figures is significant.
4. Under such optimal conditions the daily loss of Ca is 0.3 mg. per rat. Of this one-third is lost in the faeces and two-thirds in the urine.
5. The retention of P is never so efficient, even in the presence of extra Ca (as CaCO_3). The minimum daily loss observed was 1.3 mg. per rat. Of this 1.1 mg., or nearly 90 %, was in the faeces.
6. It is suggested that this faecal P is mainly bacterial P and that the relatively inefficient retention of P at sub-optimal levels of intake is due to the fixation of some of this element by intestinal bacteria.
7. There is no evidence of active excretion of either Ca or P by the large intestine of the rat.

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