

XII. THE ASSIMILATION OF THE STEENBOCK-BLACK DIET IN NORMAL AND VITAMIN D-DEFICIENT RATS WITH AND WITHOUT CAECUM

BY JAMES ROBERT MAITLAND INNES
AND RAGNAR NICOLAYSEN¹

From the Institute of Animal Pathology, University of Cambridge and the Nutritional Laboratory, University of Cambridge and Medical Research Council

(Received 19 November 1936)

THE rat, like other species, needs vitamin D for optimum health. It is generally accepted, however, that the vitamin is not necessary for the prevention of rickets in rats, provided that the diet is furnished with a well balanced Ca/P ratio; provided also that these two elements are present in the diet in a readily available form.

The lessened susceptibility of the rat to rickets compared with other species, even in absence of vitamin D, has been explained by the considerable ability of this animal to absorb ingested Ca and P [Harris, 1932]. Its cause has, however, never been investigated experimentally. In the rachitogenic rations most generally used in experiments on rats, cereals, such as maize and oats, form a regular and essential component of the diet. In the Steenbock-Black diet 76 parts are yellow corn. Both in maize and oats the essential source of P is phytin (inositolhexaphosphoric acid). The significance of this form of P in the pathogenesis of rickets has been emphasized by Bruce & Callow [1934]. Phytin is not attacked by any enzyme secreted in the digestive tract. The utilization of P from this source is therefore limited to the amount which can be split off by bacterial action. Now it is well known that parts of the ingested food may be recovered from the caecum of rabbits more than a week after the ingestion. During preliminary studies of the passage of the intestinal content through the digestive tract of the rat, a similar stagnation of ingested food was observed in the rat's caecum. Carmine ingested with the food was found to stain the content of the caecum bright red several days after ingestion. In rats starved for 24 hours the caecum is full, whereas the rest of the gut is nearly empty. This stagnation of the remnants of ingested food in the caecum will favour any bacterial action. It seemed possible therefore that the utilization of the phytin P might be favoured by this action, which would explain the better ability of the rat to absorb Ca and P. The content of the caecum is also rather acid, which naturally will tend to increase the solubility of Ca and P. A study of the Ca and P assimilation of the Steenbock-Black diet by rats with and without caecum was therefore made, both in normal and vitamin D-deficient animals.

These experiments served also a second purpose. In order to study the action of vitamin D on the secretion and absorption of Ca and P in the digestive tract, a basal diet free from Ca and P had to be used. Such a diet is deficient also in other respects and metabolism experiments with it must therefore be of very short duration, with intervals during which a more sufficient diet is given. In

¹ Rockefeller Research Fellow.

short-time metabolism experiments, the large caecum of the rat might retain previously administered food rich in Ca and P and in this way upset the results. It was therefore necessary to amputate the caecum in the rats used for such short-time metabolism experiments, but first the effect of this operation on the animals had to be studied, to see how far results obtained could be applied to intact rats. (The operations were performed by J. R. M. I.; the planning of the experiments and the analytical work by R. N.)

EXPERIMENTAL

Young rats were used, bred on stock diet until they weighed about 50 g. Half of the animals were then operated on. The caecum was amputated 3–5 mm. from the ileum and the stump closed with two layers of suture. The one important point in this operation is to use thin enough silk. Carell's artery needles with silk No. 00 can be used, but artery needles with attached silk No. 000000 are still better. The rats received milk and sugar on the day of operation and again the day afterwards. Then they were put on the rachitogenic diet for 10 days before the experiments were started.

It was found that the animals recovered quickly from the operation, kept their weight constant for about a week and then recommenced to gain in weight

Table I. *Weekly metabolism experiments on rats with and without caecum, fed on a Steenbock-Black diet with addition of 50 I.U. vitamin D daily*

Rat no.	Food intake g.	g. dry faeces per g. food	Ca intake mg.	Ca in faeces mg.	mg. Ca in faeces per g. food	Net absorbed Ca mg.	P intake mg.	P in faeces mg.	mg. P in faeces per g. food	Net absorbed P mg.	
Rats with caecum 1st week:											
1	58.0	0.108	700	507	8.7	253	151	104	1.8	47	
2	43.5	0.121	553	365	8.3	188	113	80	1.8	33	
3	49.5	0.124	630	498	10.0	130	129	105	2.1	24	
4	45.0	0.110	572	405	9.0	167	117	81	1.8	31	
5	48.0	0.113	610	390	8.0	220	125	82	1.7	43	
Rats with caecum 2nd week:											
1	59.5	0.102	758	500	8.4	258	155	134	2.3	21	
2	67.5	0.100	860	568	8.4	292	175	147	2.1	28	
3	53.0	0.100	675	485	9.2	190	138	117	2.2	21	
4	58.0	0.088	740	424	7.3	316	151	109	1.9	42	
5	51.0	0.100	650	425	8.3	215	132	112	2.2	20	
Rats without caecum 1st week:											
1	56.3	0.099	718	446	8.0	273	146	89	1.6	57	
2	56.3	0.134	718	475	8.4	244	146	100	1.8	46	
3	56.8	0.129	722	465	8.2	257	148	96	1.7	52	
4	48.8	0.134	621	430	8.8	191	127	95	1.9	32	
5	46.5	0.118	592	340	7.3	252	121	85	1.8	36	
6	56.0	0.133	713	495	8.8	218	146	100	1.8	46	
7	53.5	0.133	682	453	8.5	229	138	106	2.0	33	
Rats without caecum 2nd week:											
1	57.0	0.100	725	367	6.4	358	148	92	1.6	56	
2	54.5	0.140	695	490	9.0	205	142	116	2.1	26	
3	63.0	0.130	802	540	8.6	262	164	152	2.4	12	
4	59.0	0.123	751	500	8.5	251	154	121	2.1	33	
5	50.5	0.106	644	342	6.8	302	132	90	1.8	42	
6	60.0	0.135	755	500	8.3	255	156	142	2.4	14	
7	52.0	0.131	662	472	9.1	190	135	119	2.6	16	
					Av.	238				Av.	33
						= 34 mg.					= 4.7 mg.
						daily					daily

Table II. *Weekly metabolism experiments on rats with and without caecum, fed a Steenbock-Black diet without any addition of vitamin D*

Rat no.	Food intake g.	g. dry faeces per g. food	Ca intake mg.	Ca in faeces mg.	mg. Ca in faeces per g. food	Net absorbed Ca mg.	P intake mg.	P in faeces mg.	mg. P in faeces per g. food	Net absorbed P mg.	
Rats with caecum 1st week:											
1	48.9	0.101	622	521	10.7	101	128	122	2.5	6	
2	53.6	0.101	681	565	10.6	116	139	132	2.5	7	
3	65.5	0.105	835	728	11.1	107	172	158	2.4	14	
4	68.0	0.103	865	747	11.0	118	177	165	2.5	12	
5	54.2	0.108	690	566	10.6	124	142	129	2.4	13	
Rats with caecum 2nd week:											
1	50.2	0.102	640	580	11.6	60	132	125	2.5	7	
2	54.8	0.105	698	620	11.4	78	143	140	2.6	3	
3	70.8	0.107	901	817	11.5	84	184	175	2.5	9	
4	72.9	0.106	929	787	10.9	142	190	180	2.5	10	
5	54.0	0.108	688	620	11.5	68	140	131	2.4	9	
Rats without caecum 1st week:											
1	70.0	0.113	890	765	10.9	125	182	173	2.5	9	
2	69.8	0.125	887	754	10.8	133	182	172	2.5	10	
3	64.2	0.123	819	762	11.8	57	167	156	2.5	11	
4	62.7	0.132	800	710	11.3	90	166	160	2.6	6	
5	61.8	0.122	786	630	10.2	156	163	149	2.4	14	
Rats without caecum 2nd week:											
1	69.7	0.115	887	755	10.9	132	182	161	2.4	14	
2	68.6	0.136	874	775	11.1	99	179	170	2.5	9	
3	68.2	0.127	870	820	12.0	50	177	166	2.4	11	
4	61.3	0.127	780	640	10.5	140	159	153	2.5	6	
5	68.2	0.115	870	640	9.5	130	179	169	2.6	10	
					Av.	105				Av.	9.5
						= 15 mg.					= 1.4 mg.
						daily					daily

at the same rate as the control rats. About 100 rats in all were operated upon, roughly 10% of which died. In all cases where a post-mortem was performed an invagination of the ileum into the colon was found. No secondary dilatation of the rest of the caecum was found in rats which had been living 6-8 weeks after the operation. Later a slight dilatation was observed.

Four groups of rats were used: (1) intact rats on rachitogenic diet + vitamin D; (2) rats without caecum on rachitogenic diet + vitamin D; (3) intact rats on rachitogenic diet; (4) rats without caecum on rachitogenic diet.

The experiments performed to investigate the role of the caecum lasted two weeks in each rat. The urine was absorbed on thick blotting paper, so that faeces free from contamination could be obtained, and any food which was spilt could be recovered. The food intake in one week was measured to the nearest 0.1 g. The experimental results are given in Tables I and II.

DISCUSSION

The findings may be briefly stated as follows. (1) No difference was found in the assimilation of P and Ca as between the rats with and without caecum, either in the normal or in the vitamin D-deficient rats. This is clear from the figures which give the Ca and P in the faeces per g. food intake (see columns 6 and 10). It can therefore be concluded that the caecum has nothing to do with the greater ability of the rat to assimilate ingested Ca and P. (2) A difference was

seen, as was to be expected, in the assimilation of Ca and P as between normal and rachitic rats. (3) There is a difference in the output of dry material in the faeces as between the rats with and those without caecum, but no difference between comparable rats (see Tables I and II). The faeces excreted by the rats without caecum were only slightly more moist than the faeces excreted by the intact rats. An increased output of salts could not therefore account for the output of more dry material in the faeces excreted by the caecum-free rats. The cause of this increased excretion has not been ascertained. Considering that the chief biological processes in the caecum are of bacterial origin, it is probable that some part of the food, hardly attacked by the enzymes secreted into the digestive tract, but split up by bacterial action, is less digested by the rats without caecum. The increased output of dry material in the faeces corresponds to only 1-2% less utilization of the food in these rats. It is thus established that caecum-free rats are well suited for metabolism experiments, where the purpose is to study the Ca and P metabolism. The question of the Ca and P requirement of rachitic rats is raised by the observations recorded above. This question is dealt with in an accompanying note.

SUMMARY

The assimilation of the Steenbock-Black diet has been studied in normal and vitamin D-deficient rats with and without caecum. The absorption of Ca and P from this diet is quite unaffected by extirpation of the caecum, the only effect being a slightly decreased (1-2% less) utilization of the diet. The slow rate of passage of the intestinal contents through the caecum of the rat does not play any role in the smaller susceptibility of the rat to rickets.

My (R. N.) thanks are due to the Medical Research Council and to Dr L. J. Harris for their kind hospitality during this work.

REFERENCES

- Bruce & Callow (1934). *Biochem. J.* **28**, 517.
Harris (1932). *Lancet*, *i*, 1031.