NITROGEN, POTASSIUM, SODIUM, AND CHLORINE METABOLISM IN RICKETS, WITH SPECIAL REFERENCE TO BILIARY FISTULA RICKETS IN PUPPIES*

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Pavlov (1) discovered in 1904 that biliary fistula in dogs may lead to definite changes in the osseous system. On the basis of studies (2) on this subject which were published in 1932, we concluded that this bone disease could be considered rachitic provided the biliary fistula was performed in puppies from 6 to 7 weeks of age which had been deprived of vitamin D and sunlight from birth on throughout the period of the experiment. It is important, furthermore, that a certain rate of growth be maintained for from 4 to 6 weeks after the operation, so that the rachitic bone lesions may become manifest.

The foregoing conclusion was reached from the following facts: presence of hypophosphatemia; impaired phosphorus and calcium balance; bone changes identified as rachitic in nature by chemical, histological, and roentgenological examination; spontaneous occurrence of tetanic convulsions in one dog accompanied by hypocalcemia and increased mechanical and electrical excitability; and, finally, the curative effect of vitamin D.

In spite of this evidence which, to a great extent, was in favor of the diagnosis of rickets, there was one chief reason for doubting whether this condition might be compared, from the point of view of the metabolism, with rickets that occurs spontaneously in infants or with rickets that is induced by diet in rats; namely, the inhibited weight curve of the puppies which had been operated on. It was shown that the animals in which the gall bladder fistula had been established gained in weight for from 4 to 6 weeks after the operation, al-

* These studies were made partly in the Universitätskinderklinik, Freiburg i. Br., Germany.

though the gain was, during this period, 50 per cent less than that observed in the controls and ceased completely from 1 month to 1½ months after the operation. The control animals continued to gain for from 2 to 3 months longer. The initial gain in weight in the biliary fistula puppies was followed by a progressive loss in weight that finally led to cachexia and death.

This inhibited development, indicating a far reaching interference with the entire metabolism, is not a part of the rachitic condition. For this reason it was thought advisable, having studied the excretion of phosphates and calcium (2), to investigate also the metabolism of substances which, according to Schloss (3), do yield a normal balance in infantile rickets. Studies on puppies, infants, and rats are here reported.

Nitrogen, Potassium, Sodium, and Chlorine Metabolism in Puppies with and without Rickets Induced by Gall Bladder Fistula

The balance for nitrogen, potassium, sodium, and chlorine[1] was studied in the same puppies and for the same metabolism periods used for the calcium and phosphate excretion, already reported (2).

The experiments were done in periods of from 7 to 12 days, in three dogs (Table I, dogs 1, 5, 3) in which, 6 weeks previously, a gall bladder fistula had been established. These dogs, it will be recalled, had been deprived of sunlight and vitamin D since birth. At the time of the experiments they showed all the signs of a fully developed rachitic state such as has been referred to above. Their weight had just begun to decrease.

The same studies were made on three control animals (Table I, dogs 3, 4, 6) which, of course, had also been deprived of sunlight. Dog 3 of the control group is the same as dog 3 of the rachitic group, with the exception that 1 and 4 weeks before the second metabolism period this dog was given 50 clinical units of vigantol by mouth, for 8 consecutive days. The same dose was given also in a third 8 day period, throughout the second metabolism experiment. Rachitic bone lesions and blood chemistry changes had then become entirely normal. Dog biscuits were fed during the metabolism periods only; at other times all six dogs were given a mixed diet consisting mainly of milk, cream of wheat, potatoes, noodles, and, occasionally, meat and bone.

[1] The following chemical methods were used in the experiments: For nitrogen determination, Kjeldahl; for potassium, Kramer and Tisdall (4); for sodium, Kramer and Gittleman (5); and for determination of chlorine in urine, Mohr (6), and in feces, Sjollema and Dienske (7).

TABLE I

Nitrogen, Potassium, Sodium, and Chlorine Metabolism in Puppies with and without Rickets Induced by Gall Bladder Fistula

Dog No.	Start and end of metabolism period	Body weight at		Food	Date of operation (Bf) or treatment (V)	Degree of rickets by x-ray	Blood serum		Intake				Excretion in							
													Urine				Stool			
		Start	End				Ca	P	N	K	Na	Cl	N	K	Na	Cl	N	K	Na	Cl
Animals with Gall Bladder Fistula and Rickets																				
	1931	*kg.*	*kg.*	*gm.*	*1931*		*mg. per 100 cc.*	*mg. per 100 cc.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
1	Nov. 20 " 27	8.0	7.6	1200	Bf Oct. 8	++	11.3	3.8	34.35	2.1	0.25	1.19	26.38	3.56	0.0468	1.56	14.42	0.926	0.663	?
	1932				*1932*															
5	Mar. 30 Apr. 9	6.3	5.3	981	Bf Feb. 13	+++	9.4	4.1	22.8	0.15	0.004	1.008	21.12	3.4	0.138	1.48	6.76	0.25	0.228	0.067
3	" 26 May 9	7.1	6.5	1500	Bf " 11	+++	9.4	4.0	34.9	0.22	0.0061	1.541	18.0	4.69	0.241	2.58	6.96	0.414	0.175	0.214
Animals without Rickets																				
3	July 1 " 9	7.0	7.4	3000	Bf Feb. 11 V.* 50 units *q.d.*	0	11.0	6.8	69.8	0.45	0.012	3.082	15.05	4.73	0.258	2.62	16.35	1.253	0.301	0.38
4	May 19 " 28	8.65	8.6	2000	—	0	9.0	5.4	46.6	0.30	0.0081	2.058	19.89	3.41	0.1173	2.061	11.02	0.68	0.432	0.689
6	June 6 " 13	12.82	13.7	3000	—	0	12.0	5.8	69.8	0.45	0.012	3.082	34.4	5.54	0.347	2.76	19.48	0.984	0.386	0.284

* Detailed data concerning the vigantol medication for this dog are given in the text.

The data presented in Tables I and IV show that the gall bladder fistula puppies had a distinctly decreased retention for nitrogen, potassium, and chlorine, as compared with the control group, whereas no difference was found in the sodium balance. The extremely low sodium content of the dog biscuits might have caused the decidedly negative sodium balance found even in the control dogs; doubt is consequently cast on an interpretation of the increased sodium output in the rachitic animals.

The loss of nitrogen, potassium, and chlorine must be explained to a great extent, according to the results obtained, by an increased excretion in the urine. To consider these findings the result of an interference in intestinal absorption is, therefore, out of the question. Even the loss of calcium and phosphates, data for which have been reported in detail in a previous paper (2), cannot be traced to diminished absorption, because it was found that the ratio of phosphates in the stool and urine did not show the increase that it is a well known characteristic of the usual disturbed balance in rickets.

These results point directly to the assumption that, in spite of the fully developed rachitic picture in the gall bladder fistula dogs, the disease observed here must be considered distinctly different from that found in infantile rickets, chiefly because nothing is known as yet about a disturbed balance in infantile rickets concerning other substances than calcium and phosphate. The work done by Schloss (3) in 1916 has been the basis for this assumption. He found that the metabolism of nitrogen, potassium, and sodium in infants suffering from rickets was entirely normal. His studies, however, do not seem to have been repeated, in spite of the fact that his results are of primary importance to the pathogenetic conception of the rachitic state.

Nitrogen, Potassium, Sodium, and Chlorine Metabolism in Rickets and during the Healing of Rickets in Infants

In view of the fact that the results of the experiments on dogs, just reported, differ greatly from those stated by Schloss to have been found in infants, it was thought worth while to investigate the balance of these substances in rachitic infants and in rachitic rats. It is also true that diagnostic and therapeutic methods have become much more accurate since 1916.

Another reason for these further studies was the fact that it is still widely assumed that rachitic hypophosphatemia may be explained by a lack of intestinal absorption of calcium and phosphates (8). There are, however, so far as infantile rickets is concerned, many data (9) which cannot be brought into accord with this theory. The increased excretion of calcium and phosphates in the feces in rickets might just as well be attributed to increased excretion through the intestinal wall. The absorption theory would, on the other hand, be greatly strengthened if one were to find in rickets an increased fecal output also of substances that are not subject to reexcretion through the intestines, for instance, nitrogen, potassium, sodium, and chlorine.

The metabolism of nitrogen, potassium, and chlorine was studied in four infants in periods of 6 days,—in three of these infants while they were ill with rickets and during the process of healing brought about by administration of vitamin D (see Table II). In order to keep conditions comparable during both the rachitic and the healing periods, the same food was given throughout. Infant Me was fed half milk, half gruel, with 5 per cent of dextrimaltose added, while infants Be, We, and Toz were fed half whole, half skimmed milk, with 6 to 8 per cent karo sugar added. Charcoal was used as marker; stool and urine specimens were collected quantitatively and saved in the refrigerator in two parts after having been acidified or alkalized with H_2SO_4 or NaOH, respectively. Apart from the rickets, the infants were healthy, and had good, formed stools, with the exception of infant We in the rachitic period, when he developed loose and pasty stools. These values, therefore, are excluded from the calculation of averages (Table IV).

No difference was found for the nitrogen and potassium balances in the two groups, exceeding the possibility of experimental error. Considering the average values, there seems to be an increased output of chlorine in the rachitic infants, which, however, does not prove constant when compared in the same child in each group. Average values obtained in a small group may not be reliable enough to be the basis for any further conclusion. The distribution of nitrogen, potassium, and chlorine in stool and urine is practically identical in both groups. This is further evidence of the accidental nature of the chlorine balance in the rachitic infants.

The conclusion to be drawn from these experiments must consequently be that, in agreement with the results of Schloss (3), the metabolism of nitrogen, potassium, and chlorine in infants ill with rickets must be considered entirely normal. The sodium balance could be

TABL

Nitrogen, Potassium, Sodium, and Chlorine Metabolism in Three Infan

Name	Age	Start and end of metabolism period	Body weight at		Food	Daily treatment	Degree of rickets by x-ray	Blood serum		
								Ca		
			Start	End				Start	End	Start
										Period of
	mos.	*1933*	*gm.*	*gm.*				*mg. per 100 cc.*	*mg. per 100 cc.*	*mg. per 100 cc.*
Me	4½	Apr. 5 " 12	5510	5300	1:1, 5% 800 cc.	—	++	12.6	9.2	3.5
		1935								
Be	11	May 25 " 31	7650	7925	No. 207, 6% 1000 cc.	—	++	7.2	7.9	3.9
We	7	June 15 " 20	7025	7265	No. 207, 8% 1000 cc.	—	+	—	8.6	—
										First Heali
		1933								
Me	4¾	Apr. 18 " 25	5420	5520	1:1, 7% 800 cc.	Vigantol 30–60 units	Some healing	—	9.8	—
		1935								
Be	12	June 22 " 28	7600	7900	No. 207, 8% 1000 cc.	Cod liver oil 0.4 gm.	" "	—	9.5	—
We	7½	July 2 " 9	7310	7750	No. 207, 8% 1000 cc.	" "	" "	9.7	9.7*	4.8
Toz	9½	Apr. 30 May 6	9605	10,075	No. 207, 8% 1000 cc.	Spontaneous	" "	11.6	10.2	3.0

* Done July 15, 1935.

E II

ts Ill with Rickets and in Four Infants during the First Healing Period

P	Intake				Excretion in							
					Urine				Stool			
End	N	K	Na	Cl	N	K	Na	Cl	N	K	Na	Cl
Rickets												
mg. per 100 cc.	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*	*gm.*
3.8	13.858	2.72	0.217	3.635	10.104	2.848	0.326	2.421	0.957	0.162	0.0582	0.042
3.5	29.881	10.61	—	7.67	20.3	23.39	—	6.65	3.39	0.543	—	0.122
3.9	23.211	8.83	—	6.063	12.0	13.8	—	2.97	5.04	0.956	—	2.94
ng Period												
6.2	14.175	3.78	0.221	3.718	10.99	2.94	0.367	4.51	1.033	0.176	0.0542	0.041
5.0	33.351	12.57	—	8.89	19.88	34.68	—	1.97	4.02	0.746	—	0.268
6.1*	35.536	13.42	—	9.25	16.5	16.18	—	7.63	3.06	0.316	—	0.195
4.4	34.85	12.37	—	7.77	23.41	12.42	—	6.98	3.74	0.430	—	0.202

studied in only one case, and the results were in accord with the findings for nitrogen, potassium, and chlorine.

Nitrogen, Potassium, Sodium, and Chlorine Metabolism in Rickets and during the Healing of Rickets in Rats

The results obtained from the studies made on infants, reported above, were confirmed by the following investigation of the nitrogen, potassium, sodium, and chlorine metabolism in seventeen rats (see Table III).

Eleven rats were studied while they were in a rachitic condition. The remaining six were given 10 prophylactic units of vigantol daily,[2] beginning 4 days before the metabolism period started. At the end of the 8 day metabolism period, the bones of these six rats were almost completely healed, as shown by roentgenogram. The rats of both the rachitic and the healing groups were weaned at the age of 3 weeks, and from this time on were fed McCollum's diet 3143. The metabolism cages were built according to Schultzer (10). Carmine was used as marker.

It may be seen from Tables III and IV that the balance and distribution of nitrogen, potassium, sodium, and chlorine in stool and urine were very much the same in both groups, whether or not the rickets had been cured.

COMMENT

The results of the three experiments described lead to the conclusion that the disease which developed in puppies deprived of vitamin D and sunlight for from 4 to 8 weeks after a gall bladder fistula had been established must be considered different in nature from infantile rickets or from rickets experimentally induced in rats. So far as the bone lesions are concerned, which in all three cases developed because of a diminished content of inorganic phosphate in the blood, we are dealing with rickets in the gall bladder fistula dogs as well. This has been reported in detail in a previous paper (2). The main distinction, however, between rickets induced by biliary fistula and other forms of rickets has been found to lie in an alteration of the entire metabolism in the former, such as is by no means present in infantile rickets or in

[2] One drop of vigantol to 20 cc. of olive oil. One drop of this solution was used and administered by pipette daily to all rats except Nos. 24 and 25 which received this dose every 2nd day.

TABLE III

Nitrogen, Potassium, Sodium, and Chlorine Metabolism in Rachitic Rats and in Rats Given Rachitic Therapy

Rat No.	Time in metabolism period	Food	Treatment	Degree of rickets by x-ray	Intake				Excretion in							
									Urine				Stool			
					N	K	Na	Cl	N	K	Na	Cl	N	K	Na	Cl
Period of Rickets																
	days	*mg.*			*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
0	6	31.28	—	+	1109	64.5	—	247.3	558	33.9	—	167.3	225	2.2	—	13.5
1	6	36.2	—	+	1220	56.5	—	298	544	31.5	—	150	293	11.2	—	21.5
2	6	37.2	—	+	1258	58.7	—	309	804	57.1	—	217	214	4.5	—	10.3
3	6	29.45	—	+	985	46	—	242	677	38.4	—	177.5	156.7	3.08	—	7.26
9	8	54.2	—	++	1930	75.4	571	476	1158	78	132	344	251	7.24	32	13.95
10	8	62.3	—	++	2220	86.6	657	547	1213	86.6	171	385	354	20.4	17.3	18.9
11	8	53.7	—	++	1912	74.6	565	472	1110	61.2	155.8	352	251.8	2.48	29	7.8
13	8	35.8	—	++	1275	49.8	378	314.5	561	58.4	102.8	147.5	118	12.8	19.1	12.8
14	8	48.5	—	++	1727	—	511	426	785	—	149.3	247.8	172.8	—	19.6	14.9
15	8	57.8	—	++	1846	—	545	455	890	—	148	245	244	—	20.3	20.3
16	8	37	—	++	1318	—	390	325	553	—	82.6	153	240.2	—	30.6	20.5
Healing Period for Rats Given Rachitic Therapy																
18	8	43.7	Vigantol* 1 drop daily	0	1529	—	—	316	758	—	—	228	178.7	—	—	5.25
19	8	36.1	" "	0	1262	77	218	261	555	48.3	80.1	134.5	172.5	6.45	9.5	13.5
21	8	26.3	" "	0	921	56.2	158.5	190	499.5	42.2	57.7	95.8	133.2	4.07	6.5	4.4
22	8	40.5	" "	0	1417	—	244	292.2	508	—	71.4	128.2	204	—	7.3	10.62
24	8	39.8	Vigantol* 1 drop every 2nd day	0	1391	85	240	287.5	1056	77.1	122.4	177	234.8	2.95	6.18	8.3
25	8	42.3	" "	0	1481	90.3	255	306	1572	76.7	128	233.8	199.3	3.12	7.56	11.7

* Detailed data concerning the vigantol medication are given in the text.

TABLE IV

Summary of Average Values Obtained from Data in Tables I, II, and III

	Urine				Stool				Total			
	N	K	Na	Cl	N	K	Na	Cl	N	K	Na	Cl
Puppies:												
With gall bladder fistula and rickets:												
Excretion of intake,* *per cent*.....	72.0	2230.0	3710.0	148.0	32.0	156.0	4270.0	10.3	104.1	2386.0	7980.0	158.3
Excretion of total output, *per cent*.....	70.7	88.6	34.1	93.7	29.2	11.3	66.5	6.0				
Without rickets:												
Excretion of intake,* *per cent*.....	38.0	1120.0	2120.0	91.5	25.0	237.0	3640.0	18.2	62.8	1367.0	5772.0	109.6
Excretion of total output, *per cent*.....	58.7	82.6	38.2	84.3	41.2	17.4	61.8	15.7				
Infants:												
During period of rickets:												
Excretion of intake, *per cent*.....									77.5	167.6		91.1
Excretion of total output, *per cent*.....	88.6	96.2		98.3	11.4	3.8		1.6				
During first healing period:												
Excretion of intake, *per cent*.....									72.8	158.0		71.1
Excretion of total output, *per cent*.....	84.8	96.7		95.5	15.1	4.3		4.5				
Rats:												
During period of rickets:												
Excretion of intake, *per cent*.....									68.2	100.0	31.0	66.0
Excretion of total output, *per cent*.....	76.4	85.0	85.0	93.5	23.6	12.0	15.0	6.5				
During healing period:												
Excretion of intake, *per cent*.....									75.0	84.0	44.0	62.5
Excretion of total ouput, *per cent*.....	79.7	93.0	92.0	94.0	20.3	7.0	8.0	6.0				

* Dogs 5 and 3 received, during the metabolism period, dog biscuit from lots in which the K and Na content differed greatly from that in the dog biscuit given to dog 1. Consequently the above values for K and Na include only the data from dogs 5 and 3, for which the intakes of K and Na were comparable.

experimental rickets in the rat. The disturbance of the metabolism in biliary fistula rickets in dogs led to an inhibited gain in weight and, furthermore, to an increased excretion not only of calcium and phosphates but also of nitrogen, potassium, sodium, and chlorine. The infants ill with rickets, on the other hand—and this was found to be true also of the rachitic rats—showed an isolated disturbance in the calcium-phosphate metabolism, while the nitrogen, potassium, sodium, and chlorine were retained and excreted in an entirely normal way. Loss of calcium and phosphates in biliary fistula rickets, consequently, may possibly be only one part of a general metabolic disturbance and may lead, because it occurs in animals that are still growing, merely to what might be called a rachitic symptom complex. There is little doubt, however, that a lack of vitamin D has an important bearing upon the pathogenesis of this disease, which to a great extent proved to be curable by the administration of vitamin D. On the other hand, under the conditions described, a lack of this vitamin is not, most probably, the only cause of the entire condition.

It has been shown by Greaves and Schmidt (11) that bile is essential to the absorption of carotene. It might be found in further studies that vitamins other than A and D are involved, vitamins, for instance, that might not be absorbed or utilized in biliary fistula animals. Experiments are needed to clarify the rôles of the different causative factors, the elimination of which might finally lead to the experimental production of a purely rachitic state.

Metabolism studies of substances such as nitrogen, potassium, sodium, and chlorine, which are not subject to reexcretion through the intestinal wall—studies which led to entirely normal figures in rachitic infants as well as in rachitic rats—do not favor the so called absorption theory of Howland (8). This theory assumes that in rickets the small intestines fail to absorb calcium and phosphates normally. If it had been found that the absorption of nitrogen, potassium, sodium, and chlorine was interfered with in rickets, a failure in the absorption also of phosphates and calcium would, by analogy, become more probable. To assume, however, only an isolated failure to absorb phosphates and calcium, substances that are subject to reexcretion through the large intestine, seems rather arbitrary. Even though the discovery of the so called beryllium rickets by Kay and his coworkers (12) has estab-

lished that a failure to absorb phosphates may be one of different possible causes for the production of rickets in rats, there is thus far no conclusive evidence that this should also be the mechanism in infantile rickets[3] (9).

SUMMARY

1. Rickets developed in three puppies deprived of vitamin D and sunlight since birth, in which, at the age of 6 to 7 weeks gall bladder fistula was established. The results of studies of their bones and of the calcium and phosphate metabolism have previously been published (2). Studies on the nitrogen, potassium, sodium, and chlorine metabolism, here presented, reveal that the metabolism was greatly interfered with as compared with that in three controls without gall bladder fistula rickets. This interference, together with an inhibited gain in weight, demonstrates that the pathogenesis of biliary fistula rickets in puppies has to be considered distinctly different from infantile rickets as well as from rickets produced in rats.

2. The nitrogen, potassium, sodium, and chlorine metabolism was also studied in three rachitic and four healthy infants, and in eleven rachitic and six control rats. The balance of these substances, as well as their distribution in stool and urine, proved to be the same whether or not rickets was present.

3. The pathogenesis of biliary fistula rickets is discussed on the basis of these studies. The assumption has been made that deficiency in other vitamins than vitamin D might have a bearing upon the development of this disease. Further study with a view to possible elimination of these unspecific factors might lead to the experimental production by gall bladder fistula of a purely rachitic state.

4. The normal balance and distribution of nitrogen, potassium, sodium, and chlorine in the stool and urine of rachitic infants and rats are considered further evidence of the hypothetical nature of the so called absorption theory in infantile rickets.

[3] It might be mentioned here that the rate of intestinal absorption of carotene in two rachitic infants, determined by means of a method recently described (13), was found to be entirely normal in both infants: infant SL absorbed, on three different occasions, 78, 76, and 82 per cent of the amount of carotene in oil given by mouth, and infant BH, on two different occasions, 55 and 67 per cent.

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