IV. THE REQUIREMENT FOR VITAMIN K OF SOME DIFFERENT SPECIES OF ANIMALS

BY HENRIK DAM, FRITZ SCHÖNHEYDER AND LIESE LEWIS

From the Biochemical Institute, University of Copenhagen

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IN our earlier work on vitamin K, the blood-clotting vitamin, we have mainly studied the need of the chick for this food constituent. As reported at the 19th Scandinavian Congress of Natural Scientists, Aug. 1936 [Dam, 1936], we have recently also investigated the behaviour of a series of other animals on a diet deficient in vitamin K. The same symptoms as are seen in chicks are easily produced in ducklings and young geese, while pigeons and canaries developed the symptoms very slowly. In the group of mammals, dogs, rats and guinea-pigs did not show any signs of the disease within the time of the experiment. Rabbits in most cases refused the diet and died so early that it was impossible to draw any definite conclusion. An experiment with pigs did not lead to a definite result.

Diets. "Diet 60": caseinogen vitamin A-free 20, dried yeast 15, sucrose 62.3, salts No. 2 2.7 [Dam & Schönheyder, 1934], cod liver oil 4.

"Diet 70": the same with starch instead of sugar.

"D2" caseinogen vitamin A-free 20, dried yeast 4, salts [Hart *et al.* 1920] 4, starch 72, cod liver oil 3.

The symptoms of the disease are haemorrhages, long clotting time, high *S*-values, and as a consequence of the severe haemorrhages, low haemoglobin content. The *S*-values [Schönheyder, 1936] represent the ratio multiplied by 10 of the concentration of thrombokinas preparation (a hen's lung extract) which under fixed circumstances is required to effect clotting of the plasma in 3 min. for sick animals and normal chicks respectively.

Ducklings. 5 animals (White Peking) were placed on diet 60 from the day of hatching and 5 other animals were placed on the diet from the 18th day after hatching. The data are given in Table I, A and B.

Table I

Table 1					Haemorrhages		<i>S</i> -value	
Duckling no.	Age in days when		Weight g.	Haemo- globin (Sahli)	Time of clotting min.	In the lining of the gizzard		Else- where
	Dead	Killed						
A								
1	—	15	147	74	> 50	+	0	—
2	—	15	161	37	> 100	+	+	31,000
3	16	—	102	48	> 150	+	0	2,400
4	16	—	119	—	—	+	0	—
5	14	—	119	—	—	+	0	—
B								
6	25	—	90	—	—	+	0	—
7	—	32	201	50	5	+	(+)	—
8	—	30	140	—	—	—	—	350
9	—	27	137	—	> 50	0	0	—
10	26	—	260	—	—	+	+	26,000

Immediately after the determination of the *S*-value, duckling 8 received 0.0081 mg. per g. body weight per day of a vitamin K concentrate with 200,000 units per g. [Dam & Lewis, 1937] on 3 successive days. The *S*-value on the 4th day was found to be 12.

The lung extract used for determination of the *S*-values was tested against many samples of normal hen's plasma. The *S*-value of normal hen's plasma is 10. Three normal ducklings were found to have *S*-values 31, 40 and 18 respectively (mean value 32). The ingestion of the vitamin K concentrate has thus rendered the plasma of duckling 8 "supernormal".

Young geese. 5 animals were placed on diet 60 from the day of hatching and 5 other animals were placed on the diet from the 6th day after hatching (Table II, A and B).

Table II

Goose no.	Age in days when		Weight g.	Haemoglobin (Sahli)	Time of clotting min.	Haemorrhages		<i>S</i> -value
	Dead	Killed				In the lining of the gizzard	Elsewhere	
A								
1	—	20	230	32	120	+	0	2,200
2	13	—	150	—	—	0	+	—
3	21	—	200	42	12–15	+	+	9,000
4	18	—	230	—	—	—	—	35,000
5	4	—	69	—	—	—	—	—
B								
6	18	—	278	—	—	+	+	9,100
7	17	—	—	—	—	—	+	—
8	18	—	268	—	—	+	+	4,300
9	6	—	—	—	—	—	—	—
10	18	—	250	—	—	?	+	12,000

The *S*-values for 2 normal young geese were found to be 16 and 8.5 respectively; mean value 12. An attempt was made to cure goose 4 by means of the same concentrate as in the experiment with ducklings, but the animal died during the second operation. Young geese are very sensitive, and it is difficult to keep them alive after the operation.

Pigeons. Animals 1-3 were placed on diet 60 at the age of about 2 months, the others were placed on diet 70 from the age of 2 months. Operative removal of blood from the carotid was attempted in some cases, but did not succeed.

Table III

Pigeon no.	Days on the diet when		Weight g.	Haemoglobin (Sahli)	Time of clotting min.	Haemorrhages		<i>P</i>
	Dead	Killed				In the lining of the gizzard	Elsewhere	
1	34	34	366	85	9	0	0	
2	—	45	350	85	18	0	0	<i>P</i>
3	—	51	290	80	8	?	0	
4	—	36	101	82	10-13	+	0	
5	—	71	265	100	8-11	+	0	
6	8	—	145	—	—	0	0	
7	10	—	135	—	—	0	0	
8	12	—	191	—	2-3	0	0	

P = "symptoms resembling polyneuritis".

Canaries. 8 birds were placed on diet 60 at the age of about 2 years.

Table IV

Canary no.	Days on the diet when		Weight g.	Haemoglobin (Sahli)	Time of clotting min.	Haemorrhages	
	Dead	Killed				In the lining of the gizzard	Elsewhere
1	—	76	15	83	10	?	0
2	—	77	16	80	8	0	0
3	6	—	15	—	—	0	0
4	—	84	18	60	3	0	0
5	70	76	19	—	3	0	0
6	70	—	18	—	—	0	+
7	—	86	20	—	6½	0	0
8	—	52	17	100	1-2	+	0

* In theca cranii.

The yellow feathers of the canaries became white in the course of the experiment owing to lack of xanthophyll [Brockmann & Völker, 1934], but the yellow colour of the fat deposits persisted.

An attempt was made to operate on some of the birds in order to determine the *S*-values, but the operation appeared to be too difficult.

Mammals. Rats. 10 animals at the age of 31 days were placed on diet 60. Rats 6-11 were given 0.6 ml. lemon juice per day.

Table V

Rat no.	Days on the diet when		Weight g.	Haemoglobin (Sahli)	Time of clotting min.	Haemorrhages
	Dead	Killed				
1	—	34	142	—	2	0
2	—	34	144	—	2½	0
3	—	34	158	—	1½	0
4	—	34	134	—	2	0
5	—	34	150	—	—	0
6	—	34	174	70	2¾	0
7	—	34	163	95	2	0
8	—	34	114	—	½-4	0
9	—	34	142	75	4	0
10	—	34	150	—	2½	0

Guinea-pigs. 10 animals weighing about 250 g. were placed on diet 60 for 6 weeks. A daily supplement of 3 ml. lemon juice per animal was given. The animals were killed. No haemorrhages were observed and the clotting times were similar to those observed in the experiment with rats.

Dogs. 4 young dogs at the age of 1½-2 months were placed on diet "D2". The juice of one lemon was mixed with the drinking water (and a little sugar) for all animals together per day.

Table VI

Dog no.	Days on the diet when		Weight g.		Haemoglobin (Sahli)	Time of clotting min.	Haemorrhages
	Dead	Killed	Before experiment	After experiment			
1	11	—	850	650	—	4	0
2	—	73	1200	3650	62	1	0
3	—	73	1300	5210	63	2½-5	0
4	—	—	1400	6160	67	2½	0

The livers of dogs 3, 4, 5 were taken to dryness in a vacuum desiccator. They were found to contain on the average 12 units of vitamin K per g. dry matter. We found 40–60 units respectively in two normal dogs.

Rabbits. 5 young rabbits weighing 500–600 g. were placed on the same diet as the dogs + the juice of one lemon for all animals per day.

Table VII

Rabbit no.	Days on the diet when		Weight g.		Haemo-globin (Sahli)	Time of clotting min.	Haemorrhages + theca cranii between theca + cranii and periosteum <i>P</i>
	Dead	Killed	Before experiment	After experiment			
1	12	—	530	410	—	—	—
2	—	8	560	500	75	13	—
3	19	—	560	390	70	5	0
4	18	—	560	470	80	3–5	0
5	9	—	570	520	—	—	0

P = "symptoms resembling polyneuritis".

Pigs. 2 pigs weighing 5.2 kg. were placed on the same diet as the dogs. Each animal received a supplement of 50 mg. ascorbic acid per day in the form of tablets.

Table VIII

Pig no.	Days on the diet when		Weight kg.	Haemo-globin (Sahli)	Time of clotting min.	Haemorrhages
	Dead	Killed				
1	—	73	13.3	85	3	
2	53	—	11.8	95	2	(stomach mucosa red)

Microscopic examinations of the stomachs were carried out by Prof. Følger of the Veterinary High School, to whom the authors are indebted. The red colour of the stomach of pig 2 was due to a hyperaemia of the superficial layer but no haemorrhages could be detected.

The liver of pig 1 was found to contain about 5–10 units of vitamin K per g. dry matter as compared with 100 units for normal pigs.

DISCUSSION

The experiments show very clearly that a diet deficient in vitamin K produces the same symptoms in chicks and ducklings. The clotting power of the blood, as indicated by the *S*-values, could be rendered normal by means of a vitamin K concentrate. The symptoms developed in young geese are also exactly the same as those in chicks. Owing to the difficulty of the operative technique the curative restoration of the clotting power of the blood of young geese was not carried out.

Pigeons appear to develop the symptoms of the disease slowly and to a very mild degree. The same holds good for canaries. In one case haemorrhages in the theca cranii were, however, apparently the cause of death.

In our opinion, the haemorrhagic defects in the gizzard are, as a rule, just as good a symptom of the disease as haemorrhages in other tissues. We have, however, seen groups of animals in which the haemorrhagic defects in the lining of the gizzard did not appear in spite of the occurrence of haemorrhages in other tissues and a prolonged clotting time. This for example holds good for a group of chicks, which received fatty acids obtained by the cold saponification of hog liver fat

[Dam, 1935]. (Chicks which received fatty acids obtained by prolonged hot saponification showed the symptoms in the gizzard just as well as in other organs.) Dam & Schönheyder [1934] have drawn attention to the possibility that certain factors may render the lining of the gizzard more resistant to the haemorrhagic changes resulting from the low clotting power of the blood. Whether a labile gizzard-protecting factor may be found in the liver fat or not, the positive observation of haemorrhagic defects in the lining of the gizzard is undoubtedly a sign of insufficient vitamin K, apart from particular cases, in which other causes may have played a role.

This is mentioned because Almquist & Stokstad [1936, 2] have expressed the view that gizzard "corrosions" is a particular deficiency disease different from the vitamin K avitaminosis.

The experiments with rats, guinea-pigs and dogs show that these animals may be kept for a long time on a diet deficient in vitamin K without showing any symptoms of the disease (and thriving fairly well). The experiments with rabbits and pigs are inconclusive. The rabbits died early from starvation. The pigs thrived well in the first 6 weeks, after which the growth was irregular and diarrhoea occurred.¹

We have, in an experiment which will be described in a separate paper, been able to produce a haemorrhagic diathesis in dogs by prolonged inanition. This condition was characterized by prolonged clotting and bleeding time. The number of thrombocytes was low (7000 against the normal value 600,000). The number of cells in the bone marrow was also low.

This haemorrhagic diathesis is not believed to be a result of lack of vitamin K.

The fact that certain species of animals can apparently dispense with vitamin K in their diet, may be explained in one of three different ways: (1) The animal has no need of the vitamin, (2) the animal can synthesize the vitamin, or (3) the vitamin is synthesized by bacteria in the intestine. Almquist & Stokstad [1936] have provided evidence for the view that the bacterial synthesis occurs in the gut of the chick, and that it may be the reason why, under certain conditions, the development of the symptoms in the chick may be retarded. Further investigation on this subject must therefore be directed to discriminate between these possibilities as explanation for the results found with pigeons, canaries and the mammals. The fact that the livers of dogs and pigs reared on a vitamin K-free diet were found to contain some vitamin K, but (particularly in the experiment with pigs) apparently less than is found in livers of normal animals of these species, could be explained as a result of a limited synthesis or of the experimental period being too short.

No evidence has hitherto been provided for the indispensability of vitamin K in the food of man. Some diseases which are characterized or followed by retarded clotting power might perhaps be supposed to have, in some way or other, a relation to lack of vitamin K due to insufficient synthesis or insufficient absorption from the intestine etc.

These are: haemophilia congenitalis, grave cases of liver diseases (including intoxications influencing the liver) and infantile sprue.

Haemophilia congenitalis has been ascribed to a cellular deficiency, i.e. a deficient functioning of the thrombocytes [Howell & Cekada, 1926], but Howell [1914] has earlier stated that the cause was lack of prothrombin—the clotting component which is practically absent in vitamin K avitaminosis. This view has also recently been maintained by Patek & Taylor [1936].

¹ Prof. Hogan has recently informed us that he has seen the haemorrhagic disease in rabbits and pigs.

We have made an experiment in which a haemophilic received an alfalfa extract. The patient was a man aged 23 years, weight 71 kg. Bleeding time $2\frac{1}{2}$ min. The clotting times (determined according to Gram [1921]) before and after the treatment were 10 and 8 min. respectively. 700,000 units of the vitamin (97 g. extract) were given in the course of 9 days, corresponding to 1.1 units per g. per day. If the human haemophilic had reacted towards the ingestion of vitamin K in the same way as a K-avitaminotic chick, this dose would have rendered the clotting power of his blood normal, supposing that he could absorb the vitamin from the rather large amount of extract.

The clotting time was, as mentioned, practically unaltered, and a new haemorrhage occurred during the experiment.

SUMMARY

1. The haemorrhagic disease which is seen in chicks reared on a diet containing an insufficient amount of vitamin K has also been observed in ducklings and young geese.
2. The disease was seen to a very mild degree only or was developing very slowly in pigeons and canaries.
3. Rats, guinea-pigs and dogs could be reared for a long time on the basal diet without showing any signs of the disease.
4. Experiments with rabbits and pigs were inconclusive.
5. The reason why certain species of animals may dispense with the vitamin in their food is discussed.
6. The possible relation between K-avitaminosis and haemophilia in man is discussed.

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