CLIV. THE ANTIHAEMORRHAGIC VITAMIN OF THE CHICK.

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PREVIOUS papers [Dam, 1929; 1930; Dam and Schönheyder, 1934] deal with a deficiency disease resembling scurvy in chicks which cannot be prevented by ascorbic acid and the cause of which is ascribed to the lack of a particular antihaemorrhagic factor (or factors) in the diet. Schönheyder [1935] has shown that there is an enormous retardation of the clotting of the blood of chicks suffering from this haemorrhagic diathesis.

The nature and distribution of the antihaemorrhagic factor have now been investigated. The investigation has led to the discovery of the fact that the factor is a fat-soluble vitamin occurring in hog-liver, hemp seed, certain cereals and vegetables, and must be different from vitamins A, D and E. It is proposed to term this factor *vitamin K* (Koagulations-Vitamin in German and the Scandinavian languages).

The following groups of foods have been tested: (1) cereals and seeds, (2) vegetables, (3) animal organs, (4) different fats and oils, (5) hen's egg.

Two of the most active substances, hog-liver and hemp seed, were divided into ether-soluble and ether-insoluble fractions, and, since the active principle was found to be fat-soluble, an elaborate fractionation of hog-liver fat was carried out. The question of the identity of the antihaemorrhagic factor with already known fat-soluble vitamins has been attacked by adding large amounts of vitamins A, D and E to the basal diet.

EXPERIMENTAL.

The animals (White Leghorns) were fed the experimental diets from the day after hatching. They were, as a rule, killed at the age of about 1 month. Housing *etc.* as described by Dam and Schönheyder [1934].

The examination of the animals includes, in addition to an inspection of the gizzard and other organs for haemorrhages, a determination of the time of clotting of the blood. The haemoglobin content of the blood has also been determined because it may give some information about the loss of blood through the haemorrhages.

The haemoglobin values were determined by the Sahli haemoglobinometer; the figures indicate the haemoglobin content in percentage of that in normal human blood.

The clotting time was determined by opening the brachial vein by a slight cut and allowing 2–3 ml. blood to drop slowly, during $\frac{1}{2}$–$\frac{3}{4}$ min., into a small porcelain bowl. The clotting time is reckoned from the venepuncture until complete clotting[1].

[1] Autopsy and clotting time determinations were kindly made by Dr F. Schönheyder to whom the author is indebted. Dr Schönheyder has in the meantime developed a new technique which enables the quantitative determination of the antihaemorrhagic vitamin to be made by a curative method based on a particularly exact measurement of the clotting time [1935]. The results presented in this paper were obtained by means of the former approximate method but give fairly accurate information about the degree of sickness of the animals.

Basal diets.

60		111		124	
Caseinogen vitamin A-free	20	Caseinogen vitamin A-free	20	Ether-extracted dried hog-liver	20
Dried yeast	15	Dried yeast	40	Dried yeast	15
Salts No. 2*	2·7	Salts No. 2	2·7	Salts No. 2	2·7
Sucrose	62·3	$CaCO_3$	0·85	Sucrose	62·3
		Sucrose	36·45		
	100		100		100
Cod-liver oil	4	Cod-liver oil	4	Cod-liver oil	4

* Dam and Schönheyder [1934].

Cereals and seeds were crushed and given instead of sucrose in diet 60 (with a small amount of $CaCO_3$ added roughly to balance the excess P_2O_5 in the cereals). *Organs* were dried in a current of air at a temperature not exceeding 45° and given as a powder instead of caseinogen in diet 60. *Vegetables* were minced and mixed into the diet or given in separate food containers. *Vitamin concentrates:* "vogan" (a vitamin A preparation from Merck, Darmstadt with 120,000 International Units per ml.), "vigantol" (pure vitamin D in sesame oil, from Merck, with 15,000 I.U. per ml.) and carotene (Hoffmann La Roche, Basel) dissolved in olive oil, were given *per os* by means of a tuberculin syringe, the doses being gradually increased from week to week. Vigantol was diluted with olive oil because the day doses otherwise would have been too small for accurate measurement with the syringe.

Hog-liver fat was put at the author's disposal by the firm of Medicinalco, Copenhagen. It was, according to the statement of the manufacturer, prepared by percolation of the desiccated liver with light petroleum several times, and thereafter with ether. The solvents were removed *in vacuo*. The percolated liver was used in diet 124 after having been further extracted with ether in a Soxhlet apparatus for 3 days. Hemp seed oil was prepared by percolating the crushed seeds with ether, and the seeds were further extracted with ether for several days before they were used in the diet as "ether-extracted hemp seed". Wheat germ oil was prepared from the fresh germ which had just been dried in air at 35°. The extraction was carried out in a large Soxhlet apparatus within 8 hours, the bulk of the ether was distilled off in CO_2 and the rest removed *in vacuo*. All oils were stored in the ice-box in completely filled containers and the liver fat *in vacuo*. The oils were used within 2 months of the day of preparation. The ether was in all cases freshly distilled and tested with mercury before use. Non-saponifiable matter of liver fat was prepared by cold saponification of 100 g. of the fat in 250 ml. ether with 40 g. KOH in 250 ml. methyl alcohol for 16–24 hours, addition of water and shaking 3–4 times with more ether; the ether extracts were shaken 3 times with aqueous KOH and then with water to neutral reaction. The extraction and purification of the ether extract were carried out in one day (in the dark room). After standing overnight in the ice-box with Na_2SO_4 and filtering, the solution was concentrated (in CO_2 and then *in vacuo*) to 100 ml. On standing overnight in the ice-box a precipitate of an amorphous substance, which is soluble in ether, could be filtered off. In the experiments in which the sterol and non-sterol fractions were tested, the bulk of the sterols was crystallised from light petroleum (after evaporation of the filtrate from the readily soluble substance). The rest of the sterols were precipitated by digitonin in small excess. The digitonin precipitate was decomposed by the pyridine-ether method of Schönheimer and Dam [1933] in the cold, and the sterol prepared in this way was added to the crystallised main portion. The

filtrate from the digitonide was brought to dryness *in vacuo* and extracted with ether which takes up the "easily soluble non-sterol fraction".

The fatty acids were precipitated from the extracted soaps by sulphuric acid after evaporation of the alcohol *in vacuo*; the acids were washed with water by decantation.

In one experiment the non-saponifiable matter was divided into a "carotene" fraction and a "xanthophyll" fraction by partitioning between light petroleum and 90 % (by volume) methyl alcohol, after removing the sterols as far as possible by crystallisation.

Table I.

Chick No.	Age in days when Dead	Age in days when Killed	Weight g.	Haemoglobin (Sahli)	Time of clotting mins.	Haemorrhages In the lining of the gizzard	Haemorrhages Elsewhere
			CEREALS AND SEEDS.				
Group 75. 62 % whole wheat:							
564	—	31	127	45	—	+	0
565	—	31	318	65	—	0	0
566	—	31	240	51	—	+	0
567	—	16	87	59	—	?	0
568	—	31	217	59	—	+	0
Group 77. 62 % wheat meal:							
574	—	16	73	48	—	+	0
575	—	31	212	45	—	+	0
576	—	31	224	39	—	+	0
577	—	29	175	37	—	+	+ +
578	—	11	83	48	—	+	+
Group 79. 62 % wheat bran:							
584	—	31	107	49	—	0	0
585	—	31	139	54	—	0	0
586	—	16	55	70	—	0	0
587	—	31	151	52	—	0	0
588	—	31	133	57	—	0	0
Group 86. 62 % wheat germ:							
619	7	—	30	—	—	0	0
620	—	28	174	66	—	0	+
621	—	7	21	—	—	?	0
622	—	33	154	55	—	0	+
623	—	38	183	55	19	+	0
699	—	16	54	—	—	+	0
700	—	30	218	59	2	?	0
Group 93. 62 % oats:							
654	—	33	412	51	2	0	0
655	—	33	442	62	1	?	?
656	—	33	315	62	1	0	0
657	5	—	29	—	—	0	0
658	—	28	234	50	3½	0	+
Group 94. 62 % yellow corn:							
659	—	30	135	42	20	+	+
660	—	33	232	46	—	+	+
661	—	33	179	65	3	+	0
662	—	33	169	35	>12	+	+
663	—	33	195	55	2	+	0
Group 95. 62 % barley:							
664	—	33	285	55	½	0	0
665	—	33	274	58	1	0	0
666	—	33	284	60	½	0	0
667	—	33	225	50	1	0?	0
668	—	33	218	58	½	0	0

Table I (*cont.*).

Chick No.	Age in days when Dead	Age in days when Killed	Weight g.	Haemoglobin (Sahli)	Time of clotting mins.	Haemorrhages In the lining of the gizzard	Haemorrhages Elsewhere
CEREALS AND SEEDS (*cont.*).							
Group 96. 62 % millet:							
669	—	33	312	60	2½	?	0
670	—	33	290	61	1	0	+
671	—	33	248	61	½	0	0
672	—	33	247	60	½	0	0
673	—	33	304	65	1	+	0
Group 97. 62 % hemp seed:							
674	—	33	258	50	½	0	0
675	—	33	263	60	1	0	0
676	—	33	313	61	½	0	0
677	—	33	310	60	½	0	0
678	—	33	310	50	1	0	0
Group 98. 62 % unpolished rice:							
679	7	—	—	—	—	—	—
680	—	20	154	32	>8	+	+ +
681	—	30	145	45	>12	+	+
682	—	15	75	27	>120	+	+
683	—	13	86	40	>18	+	+
Group 146. 62 % white sunflower seed:							
928	—	31	245	55	>37	+	0
929	—	31	198	58	30	+	0
930	—	31	275	55	1½	+	0
931	—	31	300	55	100	+	+
932	—	31	260	58	5	0	+
Group 147. 62 % black sunflower seed:							
933	13	—	68	—	—	+	+ +
934	—	23	139	55	>130	0	+ +
935	—	16	77	10	>60	+	+ +
936	—	23	146	46	>120	+	+
937	—	23	169	58	>195	0	0
Group 149. 62 % soya bean:							
943	—	33	198	53	½	?	0
944	—	33	147	55	2½	0	0
945	—	33	189	49	2½	+	0
946	—	33	228	50	1	+	0
947	—	33	151	48	2½	+	?
Group 173. 62 % rye:							
1070	—	44	260	42	110	+	+
1071	—	32	220	55	9	+	0
1072	48	—	345	—	—	+	0
1073	—	56	467	54	52	+	0
1074	—	44	265	—	—	+	0
FRACTIONS OF HEMP SEED.							
Group 120. 62 % ether-extracted hemp seed:							
801	—	25	63	50	3½	+	0
802	—	24	52	25	4	+	?
803	19	—	42	—	—	+	0
804	—	9	36	62	2½	0	?
805	—	25	143	—	—	+	0
Group 132. Diet 111 + 25 % hemp seed oil:							
862	—	42	298	50	2	0	0
863	—	42	265	60	2	0	0
864	—	43	310	55	1	?	0
865	—	39	169	45	1	?	0
866	—	33	150	60	1	?	0

Table I (*cont.*).

Chick No.	Age in days when Dead	Killed	Weight g.	Haemoglobin (Sahli)	Time of clotting mins.	Haemorrhages In the lining of the gizzard	Haemorrhages Elsewhere
			FRACTIONS OF HEMP SEED (*cont.*).				
Group 111. Diet 111 without any addition:							
751	—	52	308	35	120	+	+
752	—	52	309	50	6	+	+
753	7	—	—	—	—	0	0
754	—	42	212	32	>26	+	+
755	—	19	114	36	>60	0	+
756	—	45	170	43	>60	+	+
757	—	33	148	25	>60	+	+
			VEGETABLES.				
Group 84. Diet 60 + 25 % fresh kale:							
609	—	9	30	57	—	0	0
610	—	38	218	54	—	0	0
611	—	38	279	51	—	0	0
612	—	32	100	70	—	0	0
613	—	38	200	51	—	0	0
Group 118. Diet 60 in which 15 % sugar is replaced by dried alfalfa:							
788	—	35	249	52	5½	0	0
789	—	35	204	54	4½	0	0
790	—	35	185	57	½	0	0
791	—	27	150	49	5	0	0
792	—	23	95	53	1½	+	?
Group 104. Diet 60 without any addition:							
711	—	25	70	<10	60	+	+
712	18	—	54	—	—	+	0
713	52	—	290	34	—	+	+ +
714	—	41	99	—	—	+	+ +
715	—	46	172	10	>12	+	+ +
716	—	33	88	15	10	+	+
717	46	—	158	—	—	+	+
718	—	19	48	<10	>30	+	0
719	—	27	46	<8	>500	+	0
Group 156. Diet 111 + fresh tomato *ad lib.*:							
978	—	34	190	50	1	0	0
979	—	32	225	50	1½	0	0
980	—	31	133	62	1	?	0
981	—	3	35	—	6½	0	0
982	—	34	125	52	15–3	+	0
Group 164. Diet 111 + 10 % dried orange peel (outer layer):							
1019	—	23	42	—	2½	0	0
1020	—	34	180	53	2	0	?
1021	7	—	29	—	—	0	0
1022	—	32	182	39	17	0	0
1023	—	34	131	45	1½	0	+
1049	—	25	120	50	3	0	0
1050	—	25	111	50	10–15	0	0
Group 184. Diet 111 + fresh carrot *ad lib.*:							
1131	—	23	80	48	2	+	?
1132	—	30	155	42	30	0	0
1133	—	30	148	48	3	0	0
1134	—	32	285	43	30	+	0
1135	—	32	208	—	>90	+	+

Table I (*cont.*).

Chick No.	Age in days when Dead	Age in days when Killed	Weight g.	Haemoglobin (Sahli)	Time of clotting mins.	Haemorrhages In the lining of the gizzard	Haemorrhages Elsewhere
DRIED ORGANS.							
Group 137. 20 % dried calf-brain:							
887	—	23	92	48	>30	+	+
888	—	30	81	30	>30	+	0
889	—	28	67	40	>60	+	+
890	—	19	66	10	>60	+	0
891	—	31	136	52	>60	0	0
Group 157. 20 % dried ox-muscle:							
984	—	35	130	45	>30	0	0
985	—	23	175	40	6	0	+
986	—	34	210	36	>300	?	+
987	—	24	116	46	12	+	+
988	—	35	220	40	10	0	+
Group 158. 20 % dried adrenals (ox):							
989	—	33	112	55	3½	0	0
990	—	33	112	52	12	0	0
991	6	—	22	—	>10	?	0
992	5	—	28	—	—	0	0
993	—	9	41	50	15	0	0
Group 159. 20 % dried ox-kidney:							
994	—	39	214	48	35	0	0
995	—	39	252	35	4	0	0
996	5	—	38	—	—	0	0
997	—	37	330	48	9½	0	0
998	6	—	40	—	—	0	0
1053	—	39	178	52	12	0	0
1054	—	32	187	44	23	0	0
Group 160. 20 % dried ox-lung:							
999	—	41	172	30	>200	?	+
1000	—	41	150	30	28	+	+
1001	—	29	180	25	>60	0	++
1002	40	—	170	—	—	+	+
1003	—	23	164	42	18	0	++
Group 161. 20 % dried calf-thymus:							
1004	—	35	132	40	>120	0	+
1005	—	38	191	47	60	0	0
1006	—	38	150	42	30	0	0
1007	—	38	170	51	16	+	+
1008	—	35	68	30	4½	+	0
Group 105. 20 % dried hog-liver:							
721	—	29	133	60	1	0	0
722	22	—	147	—	—	0	0
723	7	—	39	—	—	0	0
724	—	28	177	55	1	0	0
725	—	42	290	43	1	0	0
1164	—	26	140	55	1	0	0
1164*a*	—	31	251	53	16	0	0
1165	—	29	134	48	2	0	0
1166	—	31	154	50	5	0	0
1167	—	31	167	54	4½	0	0
1168	—	28	165	50	1–3	0	?
FRACTIONS OF HOG-LIVER.							
Group 124. 20 % dried hog-liver extracted with ether:							
821	—	24	150	46	20	+	+
822	—	26	153	50	10	+	0
823	—	18	93	47	>1000	+	+
824	—	27	178	50	>30	0	?
825	24	—	138	52	>60	0	+

Table I (*cont.*).

Chick No.	Age in days when Dead	Age in days when Killed	Weight g.	Haemoglobin (Sahli)	Time of clotting mins.	Haemorrhages In the lining of the gizzard	Haemorrhages Elsewhere
			FRACTIONS OF HOG-LIVER (*cont.*).				
Group 139. Diet 124 + 3 % hog-liver fat:							
897	—	48	270	48	2	0	0
898	—	47	243	45	3½	0	0
899	—	29	138	50	1½	0	0
900	—	26	120	55	4	0	?
901		45	310	48	2	0	0
Group 125. Diet 124 + 3 % fatty acids from hog-liver fat (corresponding to 4·7 % fat):							
826	—	22	132	52	>30	0	+
827	—	21	134	42	>30	+ ?	+
828	—	23	146	42	5	0	+
829	—	20	110	53	>60	0	+
830	—	21	121	38	>30	0	+
Group 126. Diet 124 + 0·37 % non-saponifiable matter from hog-liver fat (corresponding to 4·4 % fat):							
831	—	29	200	56	14	0	0
832	—	25	152	52	12	0	0
833	—	29	157	53	13	0	0
834	—	29	204	57	2½	0	0
835	—	29	188	59	4	0	0
Group 150. Diet 124 + 0·075 % of a fraction of the non-saponifiable matter sparingly soluble in ether (corresponding to 6 % fat):							
948	23	—	123	—	—	+	+ +
949	24	—	185	—	—	+	+ +
950	—	29	120	35	>60	+	+
951	25	—	120	—	—	+	+
952	—	11	55	52	7	+	+
Group 151. Diet 124 + 0·4 % sterol from hog-liver fat (corresponding to 6 % fat):							
953	—	28	165	22	60	+	+
954	22	—	151	—	—	0	+
955	—	28	136	32	75	0	+ +
956	—	20	132	30	15	0	+ +
957	—	28	105	8	>60	+	+ +
Group 152. Diet 124 + non-saponifiable matter from hog-liver fat freed from sterol and the sparingly soluble fraction (corresponding to 6 % fat):							
958	—	28	220	53	1	0	0
959	—	30	185	52	6	0	0
960	—	30	165	55	2	0	0
961	—	30	195	57	15–6	0?	0
962	—	28	230	55	1	0	0
Group 171. Diet 124 + xanthophyll fraction of the non-saponifiable matter (corresponding to 6 % fat in the first 8 days, thereafter to 12 %):							
1060	24	—	115	—	>30	+	+
1061	—	24	130	45	>45	+	0
1062	24	—	124	—	>60	+	+
1063	16	—	78	—	93	+	+
1064	—	31	120	55	140	+	0
Group 172. Diet 124 + carotene fraction of the non-saponifiable matter (corresponding to 6 % fat in the first 8 days, thereafter to 12 %):							
1065	—	30	170	55	1½	0	0
1066	—	29	185	50	4	0	0
1067	23	—	137	—	—	0	0
1068	—	31	118	55	4	0	0
1069	—	31	120	50	1½	0	0

Table I (*cont.*).

Chick No.	Age in days when Dead	Age in days when Killed	Weight g.	Haemoglobin (Sahli)	Time of clotting mins.	Haemorrhages In the lining of the gizzard	Haemorrhages Elsewhere	Vogan Total ml.	Vogan I.U.	Carotene Total mg.	Vigantol Total ml.	Vigantol I.U.
Fractions of hog-liver (*cont.*).												
Group 185. Diet 124 + 4 % hog-liver fat which had been heated on a boiling water-bath for 12 hours in contact with air (as a 1–2 mm. deep layer):												
1136	—	30	220	58	2	+	0					
1137	—	30	165	52	5½	0	0					
1138	—	30	200	50	6	?	0					
1139	—	30	220	55	1½	0	0					
1140	—	30	155	58	2	0	0					
Additions of vitamins A, D and E.												
Group 154. Diet 124 + (extra) 4 % cod-liver oil:												
968	—	27	140	29	>60	+	+ +					
969	—	27	112	32	>60	0	+					
970	—	13	84	52	>120	0	+ +					
971	—	24	125	50	>60	+	+ +					
972	—	24	105	40	>60	+	+ +					
Group 182. Diet 124 with halibut-liver oil instead of cod-liver oil (4 %) (1 g. of this oil contains 42,000 I.U. A and 2800 I.U. D):												
1121	—	27	135	35	>205	+	0					
1122	13	—	78	—	—	+	+ +					
1123	30	—	175	—	—	+	+ +					
1124	—	30	108	38	230	0	0					
1125	—	27	171	38	>230	+	0					
Group 175. Diet 124 + daily doses of "vogan" (1 ml. vogan contains 120,000 I.U. A):												
1084	—	33	164	42	18	+	0	4·25	510,000			
1085	—	33	188	43	>240	0	0	4·25	510,000			
1086	—	33	212	41	>300	+	+	4·25	510,000			
1087	15	—	50	—	—	+	+ +	1·1	132,000			
1088	—	33	211	40	>270	+	0	4·25	510,000			
Group 178. Diet 124 + daily doses of carotene (Hoffmann La Roche):										Carotene Total mg.		
1099	—	31	232	42	7½	+	0			9·6		
1100	—	28	124	38	18	+	+ +			8·6		
1101	—	31	212	48	12	+	+			9·6		
1102	23	—	175	—	—	+	+			3·05		
1103	—	28	121	38	>120	0	+ +			8·6		
Group 163. Diet 124 + daily doses of "vigantol" (1 ml. vigantol contains 15,000 I.U. D):											Vigantol Total ml.	Vigantol I.U.
1014	—	34	175	48	>120	+	+				0·21	3150
1015	—	28	152	30	>60	+	+				0·15	2250
1016	33	—	190	40	>60	+	+				0·20	3000
1017	—	34	200	52	>120	+	+				0·21	3150
1018	—	34	110	50	115	+	+				0·21	3150
Group 155. Diet 124 + 4 % wheat germ oil:												
973	—	34	218	50	60	+	+					
974	—	6	20	—	—	—	—					
975	—	42	375	50	60	0	0					
976	—	31	261	50	>60	+	+					
977	—	32	233	45	>60	0	0					
Group 148. Diet 60 + 10 % wheat germ oil:												
938	37	—	110	58	>30	+	+					
939	—	19	39	10	>60	+	0					
940	—	44	150	35	27	+	+					
941	—	44	120	35	35	+	+					
942	—	44	175	45	115	+	?					

Table I (*cont.*).

Chick No.	Age in days when Dead	Age in days when Killed	Weight g.	Haemoglobin (Sahli)	Time of clotting mins.	Haemorrhages In the lining of the gizzard	Haemorrhages Elsewhere
		ADDITIONS OF VITAMINS A, D AND E (*cont.*).					
Group 186. Diet 111 + 24 % wheat germ oil:							
1141	—	30	143	58	18	+	0
1142	15	—	50	—	1	0	0
1143	—	30	170	51	1½	+	0
1144	—	30	200	52	12	+	0
1145	—	30	160	54	2	+	0
		DIFFERENT FATS AND OILS.					
Group 110. Diet 60 + 10 % butter fat:							
746	—	31	80	<8	90	+	0
747	—	38	138	45	7	0	0
748	—	38	144	31	11	+	+
749	21	—	64	—	—	+	+
750	—	21	72	50	>60	?	0
Group 128. Diet 60 + 10 % cod-liver oil:							
842	—	20	47	20	>120	+	+
843	17	—	38	—	—	+	+
844	—	20	51	26	>60	+	+
845	—	18	70	31	>30	+	+
846	—	13	38	10	>10	+	0
Group 130. Diet 60 + 10 % soya bean oil:							
852	—	27	112	50	5	+	+
853	—	23	113	58	7	?	+
854	—	42	168	53	5	+	0
855	23	—	73	—	—	0	?
856	—	42	191	55	10	0	0
Group 131. Diet 60 + 10 % copra oil:							
857	—	13	41	<10	>30	+	0
858	—	23	74	44	120	?	0
859	12	—	37	—	—	+	0
860	—	23	97	50	27	0	0
861	—	23	82	50	5	?	0
Group 140. Diet 60 + 10 % hog-liver fat:							
902	—	28	45	30	2½	0	0
903	—	28	67	56	1	0	0
904	—	24	39	50	2½	?	0
905	—	28	89	54	2	0	0
906	—	28	68	52	4	0	0
Group 141. Diet 60 + 10 % rape oil:							
907	—	37	178	50	28	0	0
908	—	37	140	53	2½	0	0
909	29	—	50	—	—	?	+
910	—	36	130	38	>14	?	+ +
911	—	37	154	55	95	0	0
Group 142. Diet 60 + 10 % linseed oil:							
912	—	28	55	18	20	+	0
913	—	28	83	25	30	+	0
914	—	24	49	<10	>60	+	+ +
915	—	28	89	47	—	+	+
916	—	26	58	<5	>70	+	0
Group 191. Diet 124 + 10 % lard:							
1169	—	30	199	55	24	+	0
1170	—	30	153	47	>110	+	0
1171	—	30	149	48	22	?	0
1172	9	—	64	—	—	+	+ +
1173	—	30	149	42	>150	+	0

Table I (*cont.*).

Chick No.	Age in days when Dead	Age in days when Killed	Weight g.	Haemoglobin (Sahli)	Time of clotting mins.	Haemorrhages In the lining of the gizzard	Haemorrhages Elsewhere
			HEN'S EGG.				
Group 54. Diet 49* + 10 % fresh egg-yolk:							
431	—	49	241	—	—	+	+ +
432	—	52	252	—	—	0	+ +
433	—	20	64	—	—	+	+
434	—	55	452	64	—	?	0
435	—	55	352	47	—	0	+ +
Group 55. Diet 49 + 10 % fresh egg-white:							
436	—	18	43	—	—	+	+
437	—	18	42	—	—	+	0
438	—	18	54	—	—	+	+ +
439	—	18	53	—	—	+	+ +
440	18	—	57	—	—	?	+
Group 56. Diet 49 in which 20 % caseinogen was replaced by 20 % commerical desiccated egg-white:							
441	—	39	86	—	—	+	0
442	—	27	66	—	—	+	0
443	—	39	73	—	—	+	0
444	—	24	53	—	—	+	0
445	—	31	45	—	—	+	?
Group 167. Diet 60 in which 25 % sucrose was replaced by 25 % commerical desiccated egg-yolk:							
1034	—	38	270	52	½– 4½	0	0
1035	—	38	220	50	8½–28	0	0
1036	—	39	295	45	4	?	0
1037	—	38	300	50	8½–28	0	0
1038	—	38	322	55	13½	0	0
1082	—	33	322	55	7	0	0

* Dam and Schönheyder [1934].

RESULTS AND DISCUSSION.

Table I shows clearly that certain cereals and seeds will protect against the disease to a large extent whilst others are practically ineffective. Hemp seed appears to be particularly protective whilst unpolished rice, sunflower seed, yellow corn and rye are nearly valueless. The other cereals which have been tested appear to occupy a middle position.

Certain vegetables such as tomatoes, kale, orange peel *etc.* appear to have a fairly good action against the disease.

Among the organs so far tested, hog-liver is the most potent source of the active principle, while calf-brain, ox-lung and muscle are extremely poor. Ox-kidney, adrenals and calf-thymus occupy a middle position.

The hen's egg does not appear to be a very rich source, but the yolk will no doubt afford protection when given in large quantities.

All symptoms—clotting time, haemorrhages in the gizzard and other organs and haemoglobin content—are taken into consideration in estimating the condition of the animals, since at the present state of the investigation, the possibility that certain factors in the food may render the gizzard more resistant or may counteract the tendency to haemorrhage which is in turn due to the retarded clotting, has not been excluded. It is also possible that other factors or properties of the food may reduce the resistance of the gizzard or favour the

occurrence of spontaneous haemorrhages.[1] Extremely retarded growth will undoubtedly counteract spontaneous haemorrhages.

It is not probable that the low haemoglobin values which are found in animals with large haemorrhages have anything to do with lack of copper, since similar results are obtained when the diet contains 10 % marmite. 1 g. marmite has been found to contain about 0·06 mg. Cu.

The results from groups 105, 124, 139 and 140 clearly demonstrate that the active principle in hog-liver fat can be extracted with ether. Corresponding experiments with hemp seed—groups 97, 120 and 132—lead to the conclusion that the ether extract of hemp seed is protective and that the action of the extracted seed is diminished but not entirely lost, thus suggesting that the active principle of hemp seed—like the carotene of green leaves—is not altogether in association with the fat. A series of other fats and oils was tested but none of them was found to be very potent.

McFarlane *et al.* [1931] have made the observation that chicks reared on an artificial diet with ether-extracted meat meal or ether-extracted white fish meal as the source of protein suffered from a tendency to haemorrhages whilst this was not the case when the meat meal or fish meal was not extracted with ether. This is in line with the results of the present paper. McFarlane *et al.* reported, however, that they could not observe haemorrhages when caseinogen, extracted or non-extracted, was used as protein. This latter statement is incompatible with the findings of Dam [1929; 1930] and Dam and Schönheyder [1934] as well as with the present experiments in which the diets contain caseinogen.

Fractionation of hog-liver fat into fatty acids—group 125—and non-saponifiable matter—group 126—revealed the fact that the antihaemorrhagic factor is to be found in the non-saponifiable fraction, and by further fractionation—groups 150, 151 and 152—the factor was found in the easily soluble non-sterol fraction, the sterols and a fraction sparingly soluble in ether being inactive. From a solution in light petroleum the factor could not be removed by repeated shaking with 90 % methyl alcohol—groups 171 and 172. In this respect the antihaemorrhagic factor behaves like vitamin E [Olcott and Mattill, 1931] and differs from vitamin A [Wolff *et al.*, 1930]. (The paper of the last-mentioned authors deals with the partition of vitamin A between light petroleum and ethyl alcohol, but, according to a personal communication from Prof. Wolff, it has been established that methyl alcohol behaves in the same manner.)

Addition of very large amounts of vitamins A and D to the diet does not prevent the disease—group 154, extra 4 % cod-liver oil—group 182, replacement of the cod-liver oil of the basal diet by halibut-liver oil containing 42,000 I.U. of A and 2800 I.U. of D per g. whereby the amounts of vitamins A and D are raised materially—group 175, addition of the concentrated vitamin A preparation "vogan"—group 163, addition of pure vitamin D in sesame oil "vigantol".

Commercial carotene (Hoffmann La Roche), which according to the statement of the firm contains 70–80 % β- and 30–20 % α- *plus* traces of γ-carotene, does not prevent the disease when given in quantities up to 9 mg. in 1 month.

[1] Certain food components, such as desiccated hog-liver, hog-liver fat, large amounts of cod-liver oil may produce symptoms resembling polyneuritis (3 animals in group 105, 3 in group 125, 2 in group 139). This polyneuritic condition is often accompanied by multiple minute haemorrhages in the cerebellum. Such haemorrhages, however, appear to be independent of the clotting time and general haemorrhagic condition since they may appear in groups where clotting time is normal and when no haemorrhages occur in other organs. The origin of these haemorrhages and of the polyneuritic symptoms is being studied further. Polyneuritic chicks are more liable to traumatic haemorrhages than others, owing to their convulsive movements.

According to Brockman and Völker [1934] hemp seed, which is very active, is practically devoid of carotene. The conclusion is therefore justified that the antihaemorrhagic vitamin cannot be identical with vitamins A or D or with α- or β-carotene. The antihaemorrhagic vitamin bears some resemblance to vitamin E. An experiment—group 185—in which the liver fat had been heated in a boiling water-bath for 12 hours in a 1–2 mm. thick film, showed that the factor is not destroyed to any great extent under these circumstances. According to Evans [1932] vitamin E will resist such treatment. On partition between light petroleum and 90 % methyl alcohol the vitamin behaves, as mentioned, like vitamin E. It appears that a loss occurs during the saponification.

Freshly prepared wheat germ oil, however, did not protect when given at a level of 4 or 10 % in the diet, and 24 % yielded an incomplete protection—groups 155, 148 and 186. 62 % wheat germ was rather inactive—group 86. Owing to the fact that such large amounts of wheat germ oil are insufficient to prevent the disease while 3–4 % of hog-liver fat is entirely protective, it must be considered very unlikely that the antihaemorrhagic vitamin is identical with vitamin E.

It is therefore suggested that the term *vitamin K* be used for the antihaemorrhagic factor until its chemical nature has been elucidated.

Comparison of the weights of the animals in groups 105, 124 and 172 appears to indicate that vitamin K does not promote growth.

The question whether the lack of this vitamin will produce the same symptoms in other animals than chicks is under investigation.

Summary.

1. The nature and distribution of the antihaemorrhagic vitamin of the chick have been investigated.

2. The vitamin is fat-soluble and one of its richest sources is hog-liver fat, whilst cod-liver oil is practically devoid of it. The hen's egg contains the vitamin in the yolk but is apparently not an extremely rich source. In the vegetable kingdom hemp seed and certain vegetables are good sources, yellow corn, unpolished rice and sunflower seeds are very poor.

3. The vitamin occurs in the easily soluble non-sterol fraction of the unsaponifiable matter.

4. It cannot be identical with vitamin A or D because large amounts of these vitamins in the diet are completely ineffective in preventing the disease. It bears some resemblance to vitamin E with respect to solubility and resistance to heating in air, but is held to be different from E because large quantities of wheat germ and wheat germ oil do not afford complete protection against the disease.

5. It is suggested that the term *vitamin K* be used for the antihaemorrhagic factor.

6. The further chemical nature and the possible importance of the vitamin for other animals than the chick are under investigation.

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