CLXXXIII. A DEFICIENCY DISEASE IN CHICKS RESEMBLING SCURVY.

BY HENRIK DAM AND FRITZ SCHÖNHEYDER.

From the Biochemical Institute, University of Copenhagen.

(Received June 15th, 1934.)

DURING work on sterol metabolism in the chick [Dam, 1929; 1930] it was observed that chicks reared on an artificial diet consisting of vitamin A-free caseinogen 20 parts, marmite 10, soluble starch 65.5, salts [Hart *et al.*, 1920] 4.5 + varying amounts of cod-liver oil concentrates had a marked tendency to large subcutaneous and intramuscular haemorrhages and certain pathological changes in the stomach. The growth was subnormal.

It has later been shown that in the original diet the marmite may be replaced by 15 % dried brewer's yeast, the soluble starch by rice starch or sucrose, the salts by other salt mixtures (*e.g.* the special salt mixture described below), while cod-liver oil may be used instead of cod-liver oil concentrates. The low content of sterol and fat was of no importance, because the addition of cholesterol and cod-liver oil did not prevent the symptoms.

It was originally supposed that the disease was due to the lack of vitamin C in the diet, in spite of the fact that other workers have found that chicks do not need vitamin C [Plimmer and Rosedale, 1923; Hart *et al.*, 1924; 1925]. Additions of lemon juice up to 77 ml. per animal in 59 days were, however, without the slightest influence on the occurrence of the symptoms [Dam, 1930], and an attempt has now been made to give pure ascorbic acid *per os* or subcutaneously even in very large doses. The effect, however, has been absolutely negative.

When the starch in the artificial diet is replaced by a mixture of cereals, the symptoms never occur. Cereals must therefore contain a factor which prevents the tendency to haemorrhages and which is different from vitamin C.

In their work on the fat-soluble vitamin requirements of the chick, McFarlane *et al.* [1931] observed haemorrhages resembling those described in this paper but closely associated with loss of power of clotting. These authors did not observe haemorrhages, when the protein of the diet was caseinogen which had been extracted with ether. When ether-extracted white fish meal or ether-extracted meat powder was used in lieu of caseinogen the haemorrhages occurred. Rations containing fish meal and meat powder which had not been extracted with ether did not cause haemorrhages.

Description of the symptoms¹.

Haemorrhages. The haemorrhages have been observed in 60–70 % of the chicks reared on the basal diet. They set in after 11 days or later, most frequently after 15–20 days.

¹ This description is based on the autopsy of several hundred chicks of which only a small number had been used in the ascorbic acid experiments referred to in this paper.

There is no doubt that there is an important traumatic factor in the development of the haemorrhages. This explains their frequent occurrence in the left side of the breast where injections of ascorbic acid or NaCl have been given. Intramuscular and subfascial haemorrhages have been observed after pinching the muscles on the femur. Cutaneous haemorrhages occurred only in very exceptional cases. The subcutaneous haemorrhages were of different sizes varying from about $\frac{1}{2}$ cm.² up to the whole surface of the truncus.

In the majority of the cases the haemorrhages were found on breast, legs and wings. Intramuscular haemorrhages were particularly prone to occur on the legs. There was no predilection region about the knee joints. Intra-articular haemorrhages occurred only in a few cases. Sub-periosteal haemorrhages have never been seen. In some cases large haemorrhages in the abdominal cavity were found and, rather seldom, petechial haemorrhages in the liver. One chick had a large retrobulbary haemotoma. The large haemorrhages began suddenly and were followed by severe anaemia. Whether the anaemia was a mere consequence of the loss of blood has not yet been established. External haemorrhages were practically never observed.

The haemorrhages were often absorbed in the course of a few days and in some cases the animals could be reared up to the age of 2 months, at which time the experiments were interrupted.

A few animals had very large oedemas.

Changes in the mucous membrane of the stomach. The stomach of the chick consists of two parts: the cardiac cavity and the gizzard, separated from each other by an intermediary zone. At autopsy the stomach was always opened in the posterior left side by a section through the posterior main muscle in order to obtain a good survey of the inside of the gizzard. On the frontal left side and the right rear surface the secretory lining is uniformly striated, and on the upper part surrounding the orifice of the duodenum the wall of the gizzard is thin and is formed from the fibres of the musculus intermedius. The surface of the mucosa of this part shows a finely mammelated design.

Apart from very rare cases of hyperplastic inflammation in the cardiac cavity this part of the stomach never showed any pathological change. Changes were, however, constantly found in the mucous membrane of the gizzard. Macroscopically the changes appear as more or less brown coloured areas where the lining is irregularly granulated and frayed. The colour is due to haemoglobin decomposition products. The size may vary from small spots to more than 1 cm.² Small lesions often resemble tumours projecting from the surface, but ulcerated areas have also been seen. The haemorrhagic floor of the lesions may be seen from the outside when they occur on the thin-walled part of the gizzard. When large lesions occur, the contents of the gizzard may be tar-coloured and give a positive phenolphthalin reaction. Instead of the uniformly striated surface already described the surface generally was found coarsely mammelated.

The affected areas are most frequently found on the upper part of the rear wall corresponding to the musculus intermedius superior but may occur on the whole surface.

Such macroscopic changes have been found after 16 days in nearly 100 % of the chicks which were reared on the basal diets 49 and 60 and in about 50 % after 11 days.

Microscopical examinations of the affected areas have so far revealed the following facts. While the normal secretory lining is almost homogeneous so that it is somewhat difficult to see how the secretions of the individual tubular glands continue up to the surface of the lining, with arch-shaped interconnec-

tions including traces of nucleus-containing substance, the lining in the affected areas clearly reveals its structure, is less compact and has large semilunar cavities containing collections of erythrocytes. At some places the lining has quite disappeared. The haemorrhagic infiltration of the secretion may sometimes be observed in the lumen of the glands, which may be stuffed with erythrocytes in the fundus. The tunica propria itself also shows characteristic changes. The fundus of the glands is often dilated and the glandular epithelium has undergone a change which converts it from a typical cylindrical epithelium with basally placed nuclei to a shallow cubical epithelium with central nuclei.

The interglandular tissue is not much altered. It may be somewhat atrophic when the glands are strongly dilated. Signs of infiltration in the form of leucocyte-infiltration under surface epithelium very seldom occur. Hyperaemia or oedema has not been observed. Only in one single case signs of haemorrhages in the submucosa were found (a haemorrhage in process of absorption). The affections may not be considered therefore as ulcerations or inflammations in the proper sense.

The changes in the lining, the haemorrhages and the atrophic changes in the glands dominate the microscopic picture. The relationships between these symptoms will be investigated further. These conditions are illustrated by Figs. 1-4, Plate VIII.

EXPERIMENTAL.

The birds were day-old White Leghorns. They were kept indoors in cages with wire screen bottoms in order to prevent coprophagy. No food was given in the first 24 hours after hatching. Food and tap-water could be taken *ad libitum*. Injections and additions by pipette were not given before the 4th day after hatching. At autopsy all organs were macroscopically inspected, and in a large number of cases microscopic preparations (paraffin technique) were made, particularly from the gizzards.

Groups 49-53. Basal diet "49." Caseinogen, vitamin A-free, 20 parts, dried yeast 15, sucrose 60, salts (Hart, Halpin and Steenbock) 5, cod-liver oil 4 (Norwegian cod-liver oil which according to test in the vitamin laboratory of the Norwegian State contains at least 500 A and 250 D Norwegian units per g.).

Additions. *Group 49* subcutaneous injection of ascorbic acid, brought with NaHCO_3 to weak acid reaction and made isotonic with NaCl . Initial dose per animal per day 0.32 mg. ascorbic acid, the daily dose being increased from week to week. The solutions were made every second day with oxygen-free water and stored in the ice-box. The amount of liquid injected varied from 0.05 to 0.1 ml., the solution being made more concentrated according to the age of the animals.

Group 50. No addition.

Group 51. Ascorbic acid (almost neutralised) by mouth by means of a pipette. The solution consisted of ascorbic acid 30 mg., NaHCO_3 13 mg., water 1 ml., prepared with the same precautions as in group 49. Initial dose 1.5 mg. per animal per day.

Group 52. Ascorbic acid, not neutralised, subcutaneous. The solution consisted of ascorbic acid 30 mg., NaCl 45 mg., water 1 ml. Initial dose 1.5 mg.

Group 53. Ascorbic acid subcutaneous. The same solution as in group 51. Initial dose 1.5 mg.

The ascorbic acid was procured from Merck, Darmstadt. By titration against 0.001 *N* 2:6-dichlorophenolindophenol and against 0.005 *N* NaOH it was shown to contain 95-98 % of the pure substance.

Group 60. Diet: caseinogen, vitamin A-free, 20 parts, dried yeast 15, salts No. 2¹ 2·7, sucrose 62·7, cod-liver oil 4.

Subcutaneous injections of 0·9 % NaCl were given daily.

Group 66. Diet: the sucrose in diet 60 was replaced by a mixture of cereals and seeds commonly used as a constituent of ordinary chicken rations: caseinogen, vitamin A-free, 20 parts, dried yeast 15, salts No. 2 2·7, CaCO₃ 0·3, powdered mixture² 62, cod-liver oil 4.

Subcutaneous injections of 0·9 % NaCl daily.

Chick No.	Age in days when		Weight g.	Ascorbic acid mg.	Haemorrhages	Pathol. changes in the gizzard
	dead	killed				
<i>Group 49</i>						
406	—	24	83	10·7	In subcutaneous tissue on abdomen	+ +
407	—	20	71	6·8	Subcut. on right femur, subcut. and subfascial on the neck	.
408	—	24	97	10·7	Subfascial over right knee	+
409	—	18	48	5·6	0	+
410	—	27	36	14·4	Right side of breast	+
<i>Group 50</i>						
411	—	33	57	0	Subfascial right elbow	+ +
412	—	31	64	0	0	+ +
413	—	13	39	0	0	+
414	—	23	70	0	Right wrist, in the joint, intramuscular outside of right leg	+ +
415	—	25	50	0	0	+
<i>Group 51</i>						
416	—	20	57	35·7	Intramus. on right shoulder and right humerus	+
417	—	25	62	49·8	Subcut. right middle foot	+ +
418	—	18	70	29·7	Intramus. left leg	+ +
419	—	19	49	32·7	Intramus. breast, right shoulder	+ +
420	—	24	90	49·8	Subcut. left radio carp.	+ +
<i>Group 52</i>						
421	—	19	51	31·8	Large intraperitoneal	+ +
422	17	—	40	29·1	Large intraperitoneal	+ +
423	—	24	51	52·2	Subcut. and intram. left side of breast	+ +
424	—	25	44	55·8	Petechial in liver	+
425	—	13	54	19·0	Intramus. left side of breast	+
<i>Group 53</i>						
426	14	—	39	19·2	Large intraperitoneal	+
427	—	13	37	16·8	Large intraperitoneal, right side	+
428	—	7	.	.	0	.
429	—	20	82	32·7	Intramus. right leg and left side of breast	+ +
430	—	18	65	27·0	Intramus. right side of breast	+ +
<i>Group 60</i>						
471	—	20	82	0	Subcut. right breast, abdomen, petechial in liver	+
472	—	19	105	0	Intramus. and subcut. on legs	+ +
473	—	25	70	0	0	+ +
474	—	18	69	0	Subcut. right wing, leg, abdom.	+
475	16	—	64	0	Intraperitoneal left side	+ +
<i>Group 66</i>						
502	—	58	625	0	0	0
503	—	56	550	0	0	0
504	—	58	577	0	0	0
505	—	58	604	0	0	0
506	—	58	548	0	0	0

¹ CaCO₃, 2·00; MgCO₃, 0·10; Ferric citrate, 0·16; NaCl, 0·44; KI, 0·000025. (P and K is provided in yeast and caseinogen.)

² Wheat, 50; barley, 15; oats, 10; yellow corn, 10; millet, 5; hemp, 5; rice, unpolished, 5.

Fig. 1.

Fig. 4.

Fig. 2a.

Fig. 2b.

Fig. 3a.

Fig. 3b.

RESULTS AND DISCUSSION.

The experiments clearly show that ascorbic acid does not prevent the development of the syndrome. The cause of the disease cannot be a diminished absorption of vitamin C from the intestine or a diminished production of vitamin C in the organism since subcutaneous injections of ascorbic acid were ineffective. (Owing to the close clinical resemblance to scurvy it might, however, be of interest to investigate the possibility of some relation to vitamin C in other respects.)

The disease does not seem to have any relation to the salt mixture since two quite different salt mixtures can be used in the basal diet.

The only probable explanation seems to be that the cereals of diet 66 contain a factor or factors the presence of which prevents the syndrome.

Whether the haemorrhages and the changes in the gizzard are due to the lack of one and the same factor and are necessarily connected with anaemia and insufficient growth is being investigated further.

It must be considered unlikely that the factor is vitamin A or D because of the relatively large supply of cod-liver oil.

Symptoms of polyneuritis or pellagra were never observed in connection with diets 49 and 60 (but those animals on the basal diet which reached an age of more than about 20 days showed signs of leg weakness), thus rendering it unlikely that the syndrome is due to a deficiency of vitamin B₁ or B₂.

Experiments not described in this paper in which polyneuritis and pellagra were deliberately produced did not show any acceleration or aggravation of the syndrome in question.

SUMMARY.

A syndrome resembling scurvy has been observed in chicks reared on an artificial diet. The symptoms are: a tendency to large haemorrhages; certain pathological changes in the mucous membrane of the gizzard; anaemia; insufficient growth.

Ascorbic acid given by mouth or subcutaneously does not prevent the disease, the cause of which is ascribed to the lack of a factor or factors occurring in cereals.

Thanks are due to Dr K. Overgaard for assistance in providing the photomicrographs.

REFERENCES.

- Dam (1929). *Biochem. Z.* **215**, 475.
 — (1930). *Biochem. Z.* **220**, 258.
 Hart, Halpin and Steenbock (1920). *J. Biol. Chem.* **43**, 321.
 — Steenbock and Lepkowsky (1924). *J. Biol. Chem.* **60**, 341.
 — — — and Halpin (1925). *J. Biol. Chem.* **66**, 813.
 McFarlane, Graham and Richardson (1931). *Biochem. J.* **25**, 358.
 Plimmer and Rosedale (1923). *Biochem. J.* **17**, 772.

EXPLANATION OF FIGURES IN PLATE VIII.

Fig. 1. Shows a pathological gizzard.

Fig. 2. Shows (a) the normal mucous membrane of the gizzard (the secretory lining has been somewhat frayed by the preparation); (b) the same more enlarged.

Fig. 3. Shows (a) dilated glands without haemorrhages; (b) the same more enlarged.

Fig. 4. Shows glandular changes with haemorrhagic infiltration of the secretory lining.