

THE BLOOD AND BLOOD-VESSELS IN GUINEA-PIG SCURVY.*

By G. MARSHALL FINDLAY, O.B.E., M.D., Lister Fellow, Assistant to the Professor of Pathology in the University of Edinburgh.

From the Royal College of Physicians' Laboratory, Edinburgh.

SINCE Holst and Frölich (1907¹) first definitely produced scurvy in the guinea-pig, the pathology of this experimental disease has been frequently investigated. As a result of these enquiries, the theories which regarded scurvy as an infection or an intoxication have been largely discarded, since it is now generally recognised that the disease is essentially due to the absence from the diet of a definite substance—vitamin C. Nevertheless the manner in which this vitamin acts is still largely a matter of conjecture. Some animals, such as the rat, appear to be only slightly affected by its absence from the food, whilst others, such as the guinea-pig, show symptoms after a few days on a diet from which it has been excluded. The majority of those who have studied the histological changes in guinea-pig scurvy have for the most part confined their attention to the lesions in the joints and bones with the result that the changes in other organs have been somewhat neglected.

In the present investigation particular attention has been paid to the changes in the blood and vascular systems. Thirty guinea-pigs, averaging in weight from 250 to 400 grms. were employed in these experiments. Twenty-four guinea-pigs were fed on an *ad libitum* diet of oats and bran, with the addition of 60 c.c. of autoclaved milk per diem, while six guinea-pigs, as controls, were given a similar diet with the daily addition of 5 c.c. of orange-juice. Of the twenty-four guinea-pigs deprived of vitamin C, twelve were killed at intervals of forty-eight hours, beginning on the second day of the experiment, in order to investigate the gradual onset of the scorbutic condition, while twelve were allowed to develop well-marked clinical symptoms of scurvy. In the case of a guinea-pig suffering from scurvy it is usual, some six to twelve hours before death, for the animal to pass into a semi-comatose state, during which the temperature falls, while the heart and respirations gradually fail. Six guinea-pigs were therefore killed before, and six during this terminal stage. Cultures were

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made from the heart blood of all animals employed in these experiments, but in only one instance was any organism found—a gram-positive diplococcus.

1. BLOOD CHANGES.

Comparatively few observations have been made on the blood in guinea-pig scurvy. In human scurvy the majority of observers have described a secondary anæmia with a relative increase in the number of small lymphocytes, the occasional occurrence of nucleated red cells, together with well-marked poikilocytosis and polychromatophilia. Hess and Fish (1914²), however, have reported that there is very little decrease in the number of red blood corpuscles in human scurvy, while Brandt (1919³) has obtained similar results.

In the present experiments, in view of certain of the histological findings to be described later, red cell counts were made at the same time on blood obtained from the small vessels at the margin of the ear, and directly from the left heart. In order to facilitate the removal of blood from the small vessels in the ear, twelve well-grown guinea-pigs varying in weight from 350 to 400 grms. were employed. Observations were made after the appearance of definite clinical symptoms. The red cells were estimated by means of a Thoma-Zeiss hæmocytometer. The percentage of hæmoglobin present was estimated by a Haldane-Gower's hæmoglobinometer. In addition, films were made from the heart blood, stained with Leishman and used for estimating the number of red cells showing chromatophilia and those containing nuclei.

An examination of Table I. indicates that there is considerable discrepancy in the red cell counts obtained from the heart blood and from the capillaries. In the case of the heart blood the reduction in the number of red cells is in some cases considerable, while in the blood from the small vessels of the ear the decrease is comparatively small, 5,000,000 per cubic millimetre being taken as the average number of erythrocytes for a normal guinea-pig. Were it possible to obtain sufficient blood by superficial venesection from the very minute capillaries, it is probable that the decrease in the number of red cells would be even less marked. The discrepancy in the two blood counts obtained from the same animal at the same time indicates that in the later stages of guinea-pig scurvy there occurs a definite stasis of red blood corpuscles in the capillaries, a condition resembling in some degree that already noted in shock and to a lesser extent in hæmorrhage by Cannon, Fraser, and Hooper (1919⁴). Eventually, this condition of stagnation in the capillaries results in insufficient oxygenation of the tissues. It is probable that the fall in temperature which occurs shortly before the fatal termination in guinea-pig scurvy is in part responsible for the increased corpuscular content of the capillaries, but this cannot be entirely the case since the capillary

count is increased before the terminal fall in temperature. Whether similar changes occur in the blood in human scurvy is a question of some interest. It must, however, be remembered that in the guinea-pig experimental scurvy results from an absolute lack of vitamin C,—the disease is acute and invariably fatal; in man, however, there is almost always a small quantity of the anti-scorbutic substance present in the diet,—the disease is chronic and, at the present time, rarely fatal. It should be noted that the stagnation in the ear capillaries is such that the samples for counts and hæmoglobin are not absolutely comparable.

The examination of stained films from guinea-pig scurvy shows the presence of a certain number of red cells exhibiting polychromatophilia. Poikilocytosis, however, is rare, while the occurrence of nucleated red cells is by no means common. Megalocytes are also conspicuous by their absence from the peripheral blood-stream. This is of some interest since in laboratory animals a megalocytic reaction on the part of the bone-marrow is by no means uncommon as the result of hæmorrhage. So far, therefore, as the changes in the red cells show, there is no marked erythroblastic reaction in the bone-marrow in guinea-pig scurvy.

2. CHANGES IN THE VASCULAR SYSTEM.

Observations on the vascular system in guinea-pig scurvy have been scanty. Jackson and Moore (1916⁵), however, noted that certain of the veins showed marked thinning of the walls, which in certain situations appeared to have almost melted away. In addition they found both in the walls and lumen of the vessels certain minute round bodies resembling cocci. There is, however, more than a suggestion that the guinea-pigs employed by these observers were infected, since in a subsequent investigation Jackson and Moody (1916⁶) isolated from the crushed tissues of their guinea-pigs a diplococcus of low virulence.

Naked Eye and Microscopic Appearances.

In the present investigation the heart was found to be dilated in eight out of the twelve guinea-pigs dying from scurvy. In all except two cases the right side of the heart alone was dilated. Hypertrophy of the heart muscle was not encountered. A slight increase in the pericardial fluid was noted in seven instances though in only one instance did the fluid amount to as much as 1 c.c. Microscopically the muscle fibres showed loss of striation, but there was never any fatty degeneration, nor were there found any collections of granules such as those described by Hart and Lessing (1913⁷) in the degenerated heart muscles of monkeys. The interstitial connective tissue appeared œdematous in places, while the capillaries were very congested. In five cases there was definite hæmorrhagic infiltration of the heart wall.

The larger arteries and veins showed no abnormality. Hyaline degeneration, which has occasionally been described in the vessels in human scurvy, was not observed.

The capillaries and smaller venules exhibited definite pathological changes. These changes consisted of:—

Degenerative changes in the lining endothelium.

Extreme congestion.

Fine œdema of the tissues surrounding the vessels.

Hæmorrhagic areas in close relation to the capillaries.

1. Degenerative changes were extremely common in the endothelial cells lining the capillaries. At many points the cells were found to be swollen and granular, while in frozen sections stained with sudan III some of the cells showed small fat granules. Hayem (1871⁸) in examining cases of human scurvy during the siege of Paris, found fatty degeneration in the capillary walls, while Lasègue and Legroux (1871⁹) noted similar changes. Other observers have regarded these fatty changes as being due to post-mortem decomposition, but this explanation is scarcely applicable in the present instance since degenerative changes were demonstrated in the capillary endothelium of organs removed immediately after the death of the animal. Fatty and granular degeneration were noted in the capillary endothelium of the guinea-pig killed on the tenth day of the experiment.

2. Congestion of the capillaries and smaller venules was a marked feature in all organs. The degree of congestion was often such as to lead to considerable dilatation of the capillaries, a condition specially noticeable in the sinusoids of the liver and in the intertubular vessels of the kidney. As the result of pressure, degenerative changes were not infrequent in the parenchymatous cells of these organs. Actual thrombosis was not met with except in certain of the capillaries round the knee-joints.* Capillary congestion was quite well marked after twelve days feeding on a scorbutic diet.

3. In guinea-pigs dying from scurvy there was almost always noticeable a degree of fine œdema in the connective tissue in relation to the capillaries. This condition was especially well seen in the lungs, kidney and, as already described, in the heart. The œdema would appear to be due to increased transudation of fluid through the damaged capillary wall. It is thus somewhat similar to the œdema recently described by Harding (1920¹⁰) as occurring in the terminal stages of diphtheria.

4. Although prolonged search was made for ruptures in the capillary-wall, no actual breach in continuity could be found at any point. In many sections, however, there was noted an appearance

* A few observations made by M'Gowan's method on the coagulation time showed no difference between healthy guinea-pigs and those suffering from scurvy.

which strongly suggested that the red blood corpuscles were escaping by diapedesis through the intercellular cement substance. This increased permeability of the intercellular substance might presumably be brought about in two ways. Since there is considerable evidence to show that the intercellular substance is produced by the endothelial cells, it follows that degenerative processes affecting these cells also interfere with the active formation and repair of intercellular substance. In addition the degenerative processes are characterised by swelling of the endothelial cells as a result of which the intercellular substance is placed on the stretch.

A detailed account of the vascular changes in the various organs would hardly be of sufficient interest, nevertheless there are certain points in regard to the time of onset and site of the hæmorrhages, which are of importance in connection with the pathology of experimental scurvy. The earliest signs of hæmorrhage were found microscopically in a guinea-pig killed after twelve days on a scorbutic diet. In this animal minute hæmorrhagic areas were present in the muscles at the back of both knee-joints. By the fourteenth day hæmorrhages were visible to the naked eye at the knee-joints, in the axillæ and at the costo-chondral junctions. The occurrence of hæmorrhage thus followed very closely on the appearance of capillary congestion.

The site of the hæmorrhages is also of some interest. In the internal organs the hæmorrhages were all small and, except in the kidney, of microscopic dimensions. Hæmorrhagic foci were commonest in the kidney, bladder, intestine, liver, adrenal, bone-marrow and spleen in that order; they were rare in the lungs, thyroid, pancreas and pituitary, while in the central nervous system of the adult, in the thymus, lymphatic glands, testicle and ovary they were never seen. This immunity from hæmorrhage on the part of certain organs is rather remarkable. Different animals exhibited considerable variations in regard to the extent and distribution of the hæmorrhages. As a general rule those animals that survived longest on a scorbutic diet showed the most hæmorrhages. In some guinea-pigs, however, the hæmorrhages present at death were so small and so few that it was difficult to believe that the animals had died solely from the effects of secondary hæmorrhage.

In the present experiments hæmorrhage in the adrenals was only found in four animals, in all of which the cortex alone was the site of the hæmorrhage. Extensive subcapsular hæmorrhages, however, were very common. In all animals dying from scurvy marked congestion was noted both in the cortex and the medulla. The cellular changes noted by McCarrison (1919¹¹) were not well marked except in those animals which had passed into the state of unconsciousness preceding death. It is possible that the reduction of the adrenalin content noted by McCarrison in the adrenals of scorbutic guinea-pigs may have been due to the extent of these cellular changes combined with

the extreme condition of capillary stasis, since McCarrison allowed his animals to die naturally from scurvy. In the bone-marrow, hæmorrhages were frequent, both at the extremity of the long bones and in the ribs and flat bones. These hæmorrhagic foci were often so numerous and the capillary congestion so intense that little evidence of erythroblastic reaction could be detected in the bone-marrow. The absence of erythroblastic reaction possibly accounts for the small number of immature red cells found in the blood stream.

In the adult guinea-pigs examined, hæmorrhage into the central nervous system was never observed, though hæmorrhages in the meninges, were quite common. The guinea-pig killed on the sixteenth day of the experiment was found, however, to be pregnant. An opportunity was thus afforded of examining the changes produced in the offspring by a lack of vitamin C in the mother's diet. The four foetuses, which were between fifty and sixty days old, all showed hæmorrhages at the knee-joint, while in one there were extensive hæmorrhages into the substance of the medulla and cord. The hæmorrhagic foci were found not only around the central canal but scattered throughout the medulla and the cervical and dorsal regions of the cord.

TABLE I.

Red cell counts from the heart and ear capillaries, with hæmoglobin readings, in (a) acute scurvy in the guinea-pig, (b) controls.

| (a) ACUTE SCURVY. | | | | |
|-------------------|------------------------------|------------|-------------|------------|
| Guinea-pig. | Red Counts (in millions). | | Hæmoglobin. | |
| | Heart. | Capillary. | Heart. | Capillary. |
| 1 | 3·4 | 4·6 | 82 | 101 |
| 2 | 3·7 | 4·8 | ... | ... |
| 3 | 4·2 | 5·1 | ... | ... |
| 4 | 3·9 | 4·9 | 84 | 98 |
| 5 | 3·7 | 5·0 | 92 | 99 |
| 6 | 4·0 | 4·8 | 78 | 96 |
| 7 | 4·1 | 4·6 | 80 | 100 |
| 8 | 4·9 | 5·1 | ... | ... |
| 9 | 4·2 | 4·8 | 81 | 89 |
| 10 | 4·1 | 4·9 | 84 | 98 |
| 11 | 3·9 | 5·0 | 77 | 95 |
| 12 | 3·7 | 4·7 | 80 | 94 |
| (b) CONTROLS. | | | | |
| 25 | 4·8 | 4·9 | 98 | 98 |
| 26 | 5·0 | 5·1 | 96 | 98 |
| 27 | 4·7 | 5·0 | 99 | 101 |
| 28 | 4·8 | 4·8 | 95 | 96 |
| 29 | 4·6 | 4·9 | 98 | 99 |
| 30 | 4·8 | 5·2 | 97 | 99 |

TABLE II.

Number of (i.) nucleated red cells, and (ii.) red cells showing polychromatophilia for each 100 white cells counted in stained films from guinea-pigs with acute scurvy.

| Guinea-pig. | Nucleated Red Cells. | Red Cells showing Polychromatophilia. | Guinea-pig. | Nucleated Red Cells. | Red Cells showing Polychromatophilia. |
|-------------|----------------------|---------------------------------------|-------------|----------------------|---------------------------------------|
| 1 | 0 | 10 | 7 | 4 | 10 |
| 2 | 0 | 7 | 8 | 1 | 13 |
| 3 | 1 | 18 | 9 | 2 | 17 |
| 4 | 3 | 5 | 10 | 0 | 4 |
| 5 | 1 | 15 | 11 | 3 | 11 |
| 6 | 0 | 21 | 12 | 1 | 14 |

DISCUSSION.

Apart from the changes in the capillary endothelium, there have been described in guinea-pig scurvy distinct lesions in two other types of cell—the cartilage cells and osteoblasts. In the case of the cartilage cells there is noticeable, more particularly in the epiphyseal plates and at the costo-chondral junctions, a marked irregularity in the arrangement of the cells which are found to be reduced both in numbers and in size (Holst and Frölich, 1907¹). The osteoblasts are also diminished in numbers and are frequently shrivelled and atrophied. As Ingier (1913¹²) has already pointed out, these changes in the osteoblasts and chondroblasts are noticeable at points far removed from any hæmorrhagic foci and at a time when the hæmorrhages in the body are few in number or even entirely absent. The hæmorrhages cannot, therefore, be looked upon as being the cause of these degenerative changes. When animals are deprived of vitamin B there results a very profound change in all the highly specialised cells of the body, while the deficiency of vitamin C is felt more especially by the capillary endothelium, the osteoblasts and the chondroblasts. Since the function of these cells is, at least in part, concerned with the formation and repair of intercellular substance, it follows that the lack of vitamin C is largely manifested by symptoms referable to the failure to produce this substance. Thus both in young and adult animals failure in the repair of the capillary cement substance is followed by the occurrence of hæmorrhages, but in the young animal with active growth of bone the changes in the osteoblasts and chondroblasts are more pronounced than in the adult guinea-pig, in which, in acute scurvy, they may be very slight. In the blood-vessels the lack of vitamin C is first shown by degenerative changes in the capillary endothelium. The cells become swollen, with the result that the passage of the blood corpuscles is delayed. The congestion gives rise to increased transudation of fluid through the capillary-wall and finally, as the intercellular substance gradually wears out, to the

passage of red blood corpuscles into the surrounding tissues. As Bierich (1919¹³) has recently suggested in human scurvy, so in guinea-pig scurvy, the essential lesion is thus an interference with the nutrition of the capillary endothelium. The occurrence of hæmorrhages is merely incidental. The cause of death in experimental scurvy is, therefore, due, not so much to the loss of blood by hæmorrhage, but rather to the changes in the capillary endothelium as a result of which there occurs a stagnation of blood in the capillaries. The tissues are insufficiently oxygenated and death ensues.

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CONCLUSIONS.

1. The absence of vitamin C from the diet of the guinea-pig leads to swelling and degeneration of the capillary endothelium.
2. As a result of the swelling of the endothelium the flow of blood through the capillaries is retarded and extreme congestion occurs.
3. As a result of the degeneration of the endothelium the formation and repair of the intercellular substance is interfered with.
4. Small quantities of fluid pass into the surrounding tissues while at certain points red blood corpuscles escape by diapedesis.
5. The stagnation of blood in the capillaries appears to be one of the factors leading to deficient oxygenation of the tissues and thus to death.

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