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THE RELATION OF VITAMIN C TO BACTERIAL INFECTION.*

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(Plate I.)

Although the relationship of plague, pestilence, and famine has been recognised throughout the ages, it is only recently that attempts have been made to study the effects of the various dietary constituents on the resistance of the body to bacterial infection.

During the past few years intensive investigations have been carried out on diets deficient in vitamins. The experience of all observers has gone to show that animals fed on these deficient diets are far more susceptible to bacterial infection than those fed on complete diets. Up to the present, however, no explanation of this decreased resistance has been forthcoming, nor has any attempt been made to determine, even approximately, the degree of resistance possessed by animals placed on diets deficient in vitamins.

During the past eighteen months I have carried out a series of experiments on the relationship of diet to natural immunity. In the present paper an attempt is made to deal with the relationship of bacterial infection to diets deficient in vitamin C—the antiscorbutic factor.

I. Method of Investigation.

When guinea-pigs are fed on a diet of oats and bran *ad libitum* and a daily ration of 70 c.c. of autoclaved milk, they develop within a few weeks symptoms of acute scurvy, death taking place in from twenty-five to thirty-five days, though some animals manage to survive for a somewhat longer period.

Guinea-pigs suffering from acute scurvy are thus somewhat

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organs of rabbits, has come to the conclusion that the occurrence of a leucopenia coupled with the presence of myelocytes in the peripheral blood stream is of very grave import. In the scorbutic animals which suffered from pneumococcal septicæmia leucocytes with rather thick rod-shaped nuclei made their appearance in considerable numbers in the peripheral blood-stream on the second day after the injection, while in the control animals they were not noticeable in the peripheral circulation in anything like the same numbers until the fourth or fifth day. Brugsch and Schilling (1908 [12]) consider the presence of these polymorphonuclear cells with rod-shaped nuclei to be very characteristic of acute pneumonic infections.

In order still further to compare the effects of the pneumococcal infection in scorbutic and control animals, two of the latter were injected intraperitoneally with doses of 100 million pneumococci. They were killed on the third and fourth days after injection. On examination the peritoneal exudate was more extensive in the control than in the scorbutic animals. No evidence of phagocytosis of pneumococci was seen either in scorbutic or control animals, though not infrequently a polymorphonuclear leucocyte would be seen surrounded by a ring of pneumococci.

In both the scorbutic and control animals the spleen was moderately enlarged, deeply congested, soft, and friable. Microscopically there was considerable hyperplasia of the endothelial cells lining the sinuses.

The changes in the bone marrow in the scorbutic and in the control animals killed at the same time after injection showed that in the scorbutic animals dying from pneumococcal septicæmia there was a very moderate reaction on the part of the leucoblastic cells, as shown in Plate I., Fig. 6. In the control animals on the other hand, the reaction was very active: there was practically no fat to be seen in the bone marrow (Plate I., Fig. 5). In the control animals inoculated with pneumococci and then killed on the third and fourth day after injection no degenerative changes were noted in the myelocyte series of cells, but in the scorbutic animals with pneumococcal septicæmia the degenerative changes were well seen. In the myelocytes the protoplasm was more granular than usual, while karyorrhexis, karyolysis, vacuolation or even complete disappearance of the cytoplasm were all well seen. The giant cells, though not decreased in numbers, showed an irregular distribution of the nuclear chromatin which, though sometimes apparently increased in amount, was usually split up into irregular streaks or masses. In all those scorbutic animals which had died of pneumococcal septicæmia the heart muscle was found to show well-marked fatty degeneration. Neither of the two control animals killed on the third and fourth day showed any evidence of fatty change in the myocardium. When doses of 2000 million pneumococci were injected intraperitoneally death took place in all the guinea-pigs, both scorbutic and control. The scorbutic

animals, however, died in an average of 2·25 days, while the controls survived for an average period of 6·4 days. In regard to the amount of exudate into the peritoneal cavity, there was, however, in the majority of cases very little difference between the scorbutic and the control animals.

Somewhat analogous results were obtained after subcutaneous injections of pneumococci. When inoculated with 1000 million pneumococci subcutaneously all the scorbutic animals died in an average of 6·75 days, while of the controls only three out of eight died in an average period of 7·3 days.

These experiments with pneumococcus, though they require to be carried out on a far more extensive scale before entirely convincing results can be obtained, nevertheless tend to show that the scorbutic animal is as capable as the healthy animal of dealing with small numbers of pneumococci. When the dose of pneumococci is somewhat larger, *i.e.*, 1000 million, the resistance of the scorbutic animal breaks down more rapidly than that of the control. The scorbutic animal dies from an acute pneumococcal septicæmia, while the healthy animal dies after a somewhat longer period of time with a localised pneumococcal peritonitis. With still larger doses, *i.e.*, 2000 million, the resistance to the organism breaks down with about equal rapidity in healthy and scorbutic guinea-pigs. Unfortunately the method by which the tissues resist the attacks of pneumococcus is at present uncertain: the exact method of pneumococcal infection is still unknown. There is, however, some evidence to show that a toxæmia may be at work, though the results obtained after the injection of intracellular toxins from the pneumococcus have not given very concordant results. The rapidity with which fatty degeneration appears in the heart muscle and degenerative changes occur in the bone marrow in scorbutic animals suggests either that these organs were more susceptible to the action of pneumococcal toxin or that the amount of pneumococcal toxin present in the blood was in excess of that present in the blood of healthy animals similarly injected. In this connection it is of interest to note that Rosenow (1912[13]) claims to have shown that the susceptibility of guinea-pigs to the action of toxins is increased by starvation.

It has long been recognised that whatever the essential factors may be in the resistance to the pneumococcus, the activities of the leucocytes almost certainly play a part in the process. The occurrence of a definite leucocytosis in pneumonia is well recognised clinically as a sign of good prognosis. In man the polymorphonuclear leucocytes in the blood would appear to have a phagocytic action on the pneumococcus. Wadsworth (1912[14]), however, could detect no phagocytosis of pneumococci by the polymorphonuclear cells of rabbits, and in this respect the guinea-pig would appear to be similar to the rabbit. Even in the rabbit, in which presumably the pneumococci are destroyed

extracellularly, the polymorphonuclear leucocyte plays an important rôle, for Winternitz and Kline (1915 [15]) have shown that in rabbits rendered aplastic with benzol the resistance to pneumococcal infection is greatly reduced. In chronic alcoholics the resistance to pneumonia is usually low, probably as the result of the feeble leucocytosis which occurs. This feeble leucoblastic reaction is correlated with an extensive fatty degeneration of the bone marrow caused by the prolonged action of alcohol.

In guinea-pigs with chronic scurvy there are numerous areas of degeneration in the bone marrow, while in scorbutic animals dying from pneumococcal septicæmia the leucoblastic response is poor in comparison with that found in healthy control animals. It is therefore not unreasonable to conclude that the degeneration in the bone marrow in chronic scurvy may at least be one of the factors in producing a relative lowering of the resistance to pneumococcal infection.

(b) Staphylococcus aureus.

A laboratory strain of *Staphylococcus aureus* which had been grown for some months on artificial media was used for these experiments. No attempt was made to increase its virulence, which was low.

Experiment III.

Fourteen guinea-pigs of roughly the same weight, six of which were suffering from chronic scurvy, while eight were controls, were inoculated intraperitoneally with suspensions of the organism in normal saline. The results are shown in Table VI.

TABLE VI.

The Effects of Intraperitoneal Injections of Staphylococcus aureus *in Guinea-Pigs.*

No. of Animal.	Condition.	Weight in Grams.	No. of Staphylococci in millions.	Period of Survival in days.	Remarks.
39	Scorbutic .	255	5,000	...	Killed after 10 days; no peritonitis.
40	,, .	268	5,000	...	...
41	,, .	290	10,000	...	Killed after 10 days; no peritonitis.
42	,, .	275	10,000	...	...
43	,, .	244	20,000	5	Peritonitis. Staphylococcus from exudate.
44	,, .	252	20,000	6	Do. do.
45	Non-scorbutic .	247	5,000	...	Killed after 10 days; no peritonitis.
46	,, .	254	5,000	...	...
47	,, .	225	10,000	...	Killed after 10 days; no peritonitis.
48	,, .	285	10,000	...	...
49	,, .	296	20,000	...	Killed after 10 days; no peritonitis.
50	,, .	302	20,000	...	...
51	,, .	295	50,000	3	Peritonitis. Staphylococci present.
52	,, .	260	50,000	3	Do. do.

These observations show that, as in the case of the pneumococcus, both the scorbutic and healthy control guinea-pigs are capable of destroying considerable numbers of staphylococci. With intraperitoneal injections of 20,000 million staphylococci both scorbutic animals died with marked symptoms of peritonitis, though no organism could be cultured from the heart blood. The peritoneal exudate which was only moderately rich in leucocytes contained chiefly polymorphonuclear cells and mononuclear leucocytes in various stages of degeneration. The spleen was enlarged and deep red in colour. Microscopically there was evidence of a reaction to infection.

Similar changes were found in the healthy control animals which had received intraperitoneal injections of 50,000 million. In the animals, both scorbutic and control, which were killed ten days after the injection, smears from the peritoneal cavity showed only the presence of a few mononuclear cells, some of which contained the remains of partially digested polymorphonuclear cells. The spleen was normal both on naked-eye and microscopic examination. Cultures both from the peritoneum and heart blood were sterile. Two other healthy guinea-pigs, Nos. 53 and 54, were also inoculated intraperitoneally with 20,000 million staphylococci. They were killed on the fifth and sixth days after inoculation respectively. In both there was only a very small amount of fluid in the peritoneal cavity and on microscopical examination the cellular exudate was almost exclusively mononuclear in character.

Total and differential leucocyte counts were made in the case of all animals inoculated with the staphylococci. The findings in the case of a scorbutic animal—No. 43—which died after inoculation with 20,000 million staphylococci are contrasted in the table with those found in a healthy animal—No. 45—which recovered after the same treatment. (See Table VII.)

In the case of both animals there was a primary leucopenia following the inoculation, while later a leucocytosis occurred. This leucocytosis in the case of the scorbutic animal finally gave way to a secondary leucopenia associated with the presence of myelocytes in the blood stream, together with dead polymorphonuclear leucocytes and a considerable number of polymorphonuclear cells with the rod-shaped nuclei previously described as occurring after fatal injections of pneumococci.

The changes in the bone marrow of the guinea-pigs which died as a result of the staphylococcal injections were very similar to those described by Muir (1901 [16]) in rabbits dying after large intraperitoneal injections of staphylococci. The leucoblastic reaction in the bone marrow was only moderate in amount and much less marked than in the control animals inoculated with the same number of organisms. In the bone marrow of the control animals—Nos. 55 and 56—there were no signs of degeneration in the leucoblastic cells, while the

scorbutic animals showed definite evidence of degeneration both in the myelocytes and in the megakaryocytes. In one of the scorbutic animals which died from the staphylococcal infection the heart showed marked fatty degeneration.

TABLE VII.

Total and Differential Leucocyte Counts in Scorbutic and Healthy Guinea-Pigs Inoculated Intraperitoneally with 20,000 *million* Staphylococci.

Date.	Total.	Polymorphonuclears.	Eosinophils.	Basophils.	Mononuclears.	Lymphocytes.	Myelocytes.	Dead.
Guinea-Pig No. 43—Scorbutic.								
Before injection	8,180	42	4	1	7	46	...	...
3 hours after injection	4,060	46	0	2	4	48	...	...
1 day after .	10,150	55	1	3	8	33	...	...
2 days after .	14,680	57	4	2	9	27	...	1
3 ,, .	13,430	50	5	1	9	34	...	1
4 ,, .	12,500	60	1	0	6	32	1	1
5 ,, .	3,200	40	1	2	8	46	1	2
Guinea-Pig No. 45—Non-Scorbutic.								
Before injection	9,560	43	1	1	2	53	...	...
3 hours after injection	3,750	43	0	2	6	49	...	...
1 day after .	17,180	69	0	0	3	28	..	...
2 days after .	10,250	44	0	3	8	45	...	...
3 ,, .	12,120	34	1	1	6	58	...	...
4 ,, .	13,400	26	1	2	9	60	...	...
5 ,, .	10,250	36	2	1	6	55	...	...

These experiments, though less extensive and less suggestive than those with the pneumococcus, nevertheless tend to confirm the suggestion that the scorbutic guinea-pig is more susceptible to bacterial infection than the healthy animal.

(c) Streptococcus hæmolyticus.

A strain of hæmolytic streptococcus obtained from the Lister Institute was used for the injections in this experiment.

As in the case of the pneumococcus and staphylococcus, the lowering of the resistance to the streptococcus due to the scorbutic condition was very slight. The results obtained with injections of 100 and 200 million were identical both in the scorbutic and healthy animals. With doses of 500 million, however, the scorbutic animals died while the controls recovered. For comparison with the scorbutic animals two healthy guinea-pigs—Nos. 75 and 76—were inoculated intraperitoneally with 500 million streptococci and were then killed on the third and fourth days after the inoculation. A point of some interest in the

Experiment IV.

The injections were all given intraperitoneally. Ten scorbutic guinea-pigs and ten healthy controls of roughly similar weight were employed. The results obtained are shown in Table VIII.

TABLE VIII.

The Effects of Intraperitoneal Injections of Streptococcus hæmolyticus *in Scorbutic and Healthy Guinea-Pigs.*

No. of Animal.	Condition.	Weight in Grams.	No. of Strepto-cocci in millions.	Period of Survival in days.	Remarks.
55	Scorbutic .	265	100	...	Killed after 10 days; no peritonitis.
56	,, .	280	100	...	...
57	,, .	245	200	...	Killed after 10 days; no peritonitis.
58	,, .	302	200	...	...
59	,, .	255	500	3	Small amount of peritoneal exudate. Streptococci from peritoneum and heart blood.
60	,, .	260	500	2	Do. do.
61	,, .	284	1000	4	Do. do.
62	,, .	240	1000	2	Do. do.
63	,, .	245	2000	3	Do. do.
64	,, .	259	2000	3	Do. do.
65	Non-scorbutic .	240	100	...	Killed after 10 days; no peritonitis.
66	,, .	276	100	...	...
67	,, .	308	200	...	Killed after 10 days; no peritonitis.
68	,, .	315	200	...	...
69	,, .	254	500	...	Killed after 10 days; no peritonitis.
70	,, .	260	500	...	...
71	,, .	252	1000	3	Small amount of peritoneal exudate. Streptococci from peritoneum and heart blood.
72	,, .	235	1000	4	Do. do.
73	,, .	246	2000	2	Do. do.
74	,, .	259	2000	2	Do. do.

post-mortem examination of the scorbutic guinea-pigs dying from streptococcal peritonitis was the number of petechial hæmorrhages which were found on the surface of the peritoneum, pleura and round the knee-joints. These hæmorrhagic foci were all quite recent. As was previously mentioned in discussing the pathological changes in chronic scurvy, it is not common to find numerous hæmorrhages in the chronic form of the disease in the guinea-pig. These hæmorrhagic foci must, therefore, in all probability be looked upon as being due to the action of the streptococcal toxin on the endothelium already weakened by the deficiency of vitamin C. Whereas all the scorbutic guinea-pigs which died from the effects of the streptococcus showed these hæmorrhages, only one of the controls—No. 72—showed a few hæmorrhagic foci in the peritoneum. The changes in the peripheral

blood stream were very similar to those described in the case of the pneumococcus and staphylococcus. One of the scorbutic animals—No. 61—showed a very extensive gelatinous degeneration of the bone marrow, which, though in part due to the chronic deficiency of vitamin C, was largely due to the action of the streptococcus. The reaction of the bone marrow in guinea-pigs Nos. 59 and 60 was much less than that in the healthy animals—Nos. 75 and 76—which also received an injection of 500 million streptococci, while the degenerative changes in the bone marrow were also far more evident in the scorbutic than in the controls.

(d) Bacillus coli.

A stock laboratory strain of *Bacillus coli* was used for this series of inoculations. The same technique was used as in the previous experiments.

Experiment V.

Twelve guinea-pigs, six scorbutic and six control animals, were inoculated intraperitoneally with varying doses of the bacillus. The results are shown in Table IX.

TABLE IX.

The Effects of Intraperitoneal Injections of Bacillus coli *in Healthy and Scorbutic Guinea-Pigs.*

No. of Animal.	Condition.	Weight in Grams.	No. of *B. coli* in millions.	Period of Survival in days.	Remarks.
77	Scorbutic .	310	200	...	Killed 10 days later; no peritonitis.
78	,, .	345	200	...	...
79	,, .	260	700	3	Peritonitis. *B. coli* from heart blood.
80	,, .	352	700	3	Do. do.
81	,, .	330	1000	2	Do. do.
82	,, .	326	1000	1·5	Do. do.
83	Non-scorbutic .	315	200	...	Killed 10 days later; no peritonitis.
84	,, .	320	200	...	...
85	,, .	345	700	...	...
86	,, .	312	700	...	...
87	,, .	332	1000	2	Slight peritoneal exudate. *B. coli* from heart blood.
88	,, .	365	1000	2	Do. do.

With *Bacillus coli* also the scorbutic animals exhibited rather less resistance than the controls. In the scorbutic animals that died after injection with 700 million organisms there was a considerable amount of fibrinous exudate into the peritoneal cavity, whereas in those animals which succumbed more rapidly, the exudate was fluid and small in amount. As in the case of the scorbutic animals with streptococcal infection, the scorbutic guinea-pigs with *Bacillus coli* infections showed numerous hæmorrhagic areas, while the control animals showed none.

The changes in the leucocytes of the peripheral blood stream were similar to those obtained with the other organisms previously described.

Degenerative changes were especially well marked in the bone marrow of the scorbutic guinea-pigs.

IV. Discussion.

The result of these experiments with four species of bacteria seems to show that guinea-pigs fed on a diet deficient in vitamin C succumb to a smaller infecting dose of bacteria than animals fed on a complete diet. The symptoms of toxæmia are manifested more rapidly in scorbutic than in control guinea-pigs either because the tissues, especially the heart, are more susceptible to the action of bacterial toxin or because in scorbutic animals there is more toxin formed by the bacteria as a result of some rupture in the defence mechanism of the body. It has long been known that degeneration in the hæmopoietic bone marrow is associated with a reduced resistance to bacterial infection. In chronic scurvy there is present such a degeneration in the bone marrow. It therefore seems not improbable that the lesion in the bone marrow may be at least one of the factors in the reduction of the resistance to bacterial infection exhibited by animals with chronic scurvy.

In conclusion, I wish to express my gratitude to Professor J. Lorrain Smith for much assistance and advice, and to Lieut.-Col. M'Kendrick, Superintendent of the Royal College of Physicians' Laboratory, Edinburgh. Part of the expenses of this research were defrayed by a grant from the Moray Trust.

Conclusions.

1. Definite lesions occur in the bone marrow of guinea-pigs both in acute and chronic scurvy.
2. Guinea-pigs with chronic scurvy, though showing few clinical symptoms, are less resistant to bacterial infection than control animals.
3. This lowered resistance may in part be due to the degenerative changes and feeble leucoblastic reaction seen in the bone marrow of chronic scurvy guinea-pigs.

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DESCRIPTION OF PLATE I.

Fig. 1.—Bone marrow of normal guinea-pig, showing proportion of fat and hæmopoietic cells. Hæmat. and Eos. C.B. × 80.

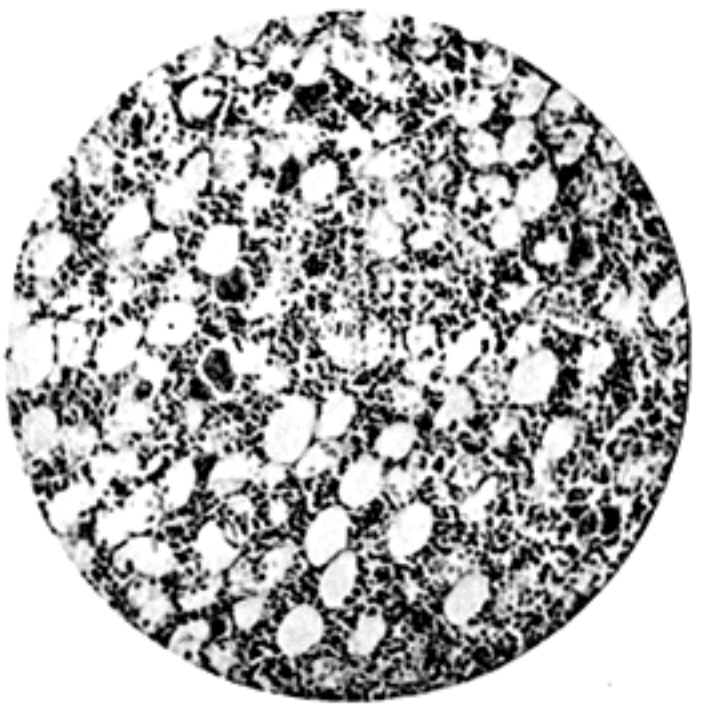
Fig. 2.—Bone marrow of guinea-pig with acute scurvy, showing acute congestion. Hæmat. and Eos. C.B. × 80.

Fig. 3.—Bone marrow of guinea-pig with chronic scurvy. Fibrosis. Hæmat. and Eos. C.B. × 80.

Fig. 4.—Bone marrow of guinea-pig with chronic scurvy. Gelatinous degeneration. Hæmat. and Eos. C.B. × 80.

Fig. 5.—Bone marrow of normal guinea-pig with pneumococcal peritonitis: well-marked leucoblastic reaction. Hæmat. and Eos. C.B. × 80.

Fig. 6.—Bone marrow of guinea-pig with chronic scurvy and pneumococcal peritonitis: feeble leucoblastic reaction. Hæmat. and Eos. C.B. × 80.



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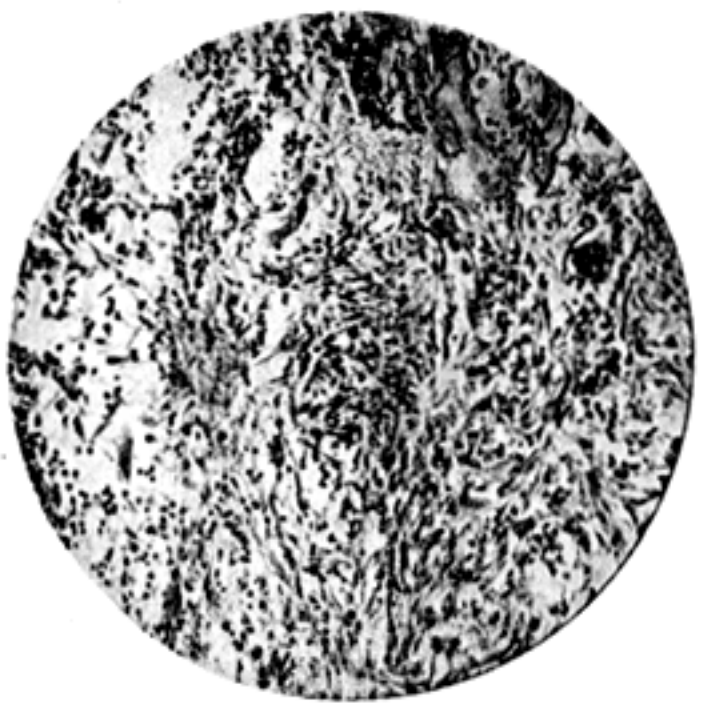

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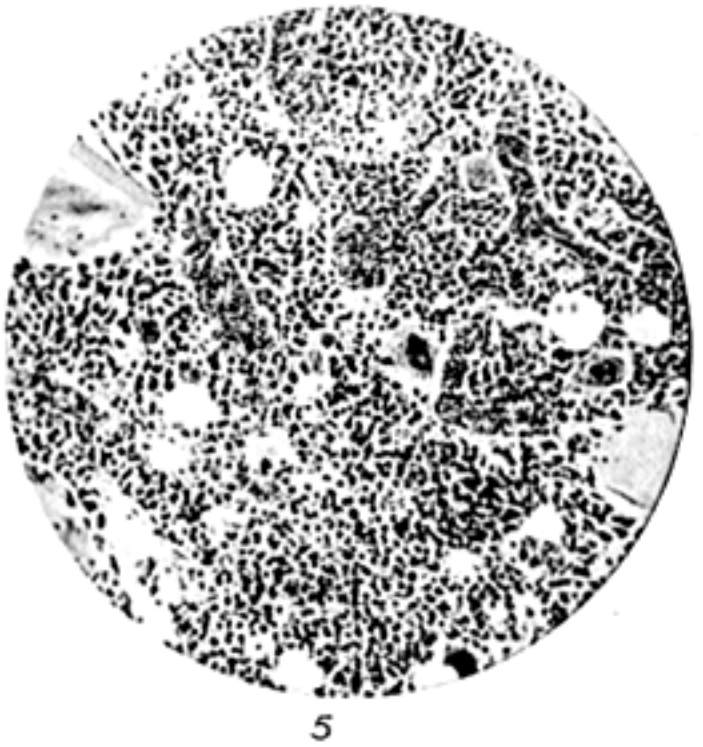

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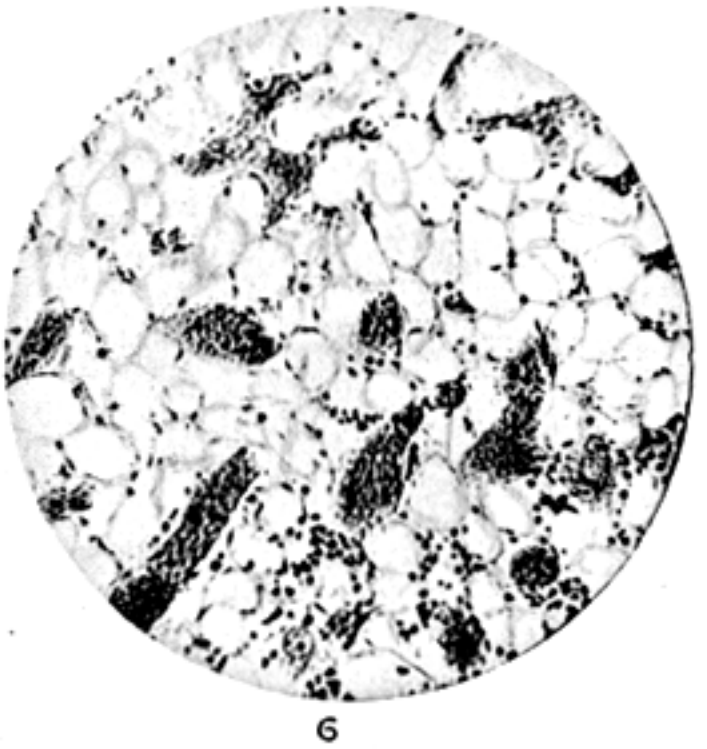

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unsuitable for testing the power of resistance to bacterial infection, since their hold on life is in any case so precarious that it is almost impossible to determine whether death is due to the superadded bacterial infection or to the lack of vitamin C in the food.

In order completely to protect a guinea-pig from scurvy, it is usual to add a daily dose of at least 5 c.c. of orange juice to the oats, bran, milk diet. When, however, a smaller quantity of orange juice, such as 2 c.c., is given every third day, the animal passes into a condition of chronic scurvy. From the clinical point of view, in animals with chronic scurvy, symptoms are practically non-existent, though close observation shows that the animals are less active than usual, and, in addition, fail to increase in weight. In the absence of definite clinical symptoms, chronic scurvy in the guinea-pig is not unlike the form of latent scurvy described by Hess (1917[1]) in children in whom nothing is to be noted except failure of growth and some indefinite malaise. Although guinea-pigs may be maintained in this condition of chronic scurvy for many weeks, they eventually die, usually from some intercurrent bacterial infection.

Before actually investigating the reaction to bacterial infection in chronic scurvy, a detailed examination was carried out of the blood and hæmopoietic organs in guinea-pigs fed on a diet deficient in vitamin C.

II. The Blood and Hæmopoietic Organs in Chronic Guinea-Pig Scurvy.

(*a*) *The Blood.*—Examination of the various cellular elements of the blood in acute guinea-pig scurvy has not up to the present shown any very striking or constant change.

Practically no observations have been made on the blood in guinea-pigs suffering from chronic scurvy.

An analysis of the blood findings in twelve cases of chronic guinea-pig scurvy is shown in Table I. It will be seen that in some instances there was a slight reduction in the number of red blood corpuscles, though the average red count was 4,620,000 per cm. Estimations of the blood platelets, using the method described by Bedson (1922[2]), showed no reduction in the proportion of platelets to red cells.

There is a very slight reduction in the number of leucocytes as compared with animals of similar weight, while in comparison with guinea-pigs of the same age which have lived on a full diet and have therefore shown a definite increase in weight, the leucopenia is more pronounced. The differential leucocyte count shows practically no deviation from the normal, thus differing from the blood findings in infantile scurvy, in which, according to Hess and Fish (1914[3]), there is usually a relative lymphocytosis.

TABLE I.

Blood Changes in Guinea-Pigs with Chronic Scurvy.

No. of Animal.	Weight in Grams.	Red Cells.	Ratio of Platelets to Red Cells.	Leucocytes.	Polymorphs.	Eosinophils.	Basophils.	Lymphocytes.	Mononuclears.
1	340	5,180,000	6·0	7,810	51	1	1	46	2
2	347	4,350,000	7·1	7,500	52	0	0	44	4
3	310	4,220,000	7·3	9,680	48	2	1	46	3
4	330	5,060,000	7·8	9,370	43	4	0	45	8
5	304	5,240,000	6·9	8,180	42	4	1	46	7
6	345	4,600,000	7·3	6,540	42	1	1	53	3
7	362	3,820,000	7·7	7,808	28	0	0	67	5
8	320	4,200,000	6·1	8,210	54	2	0	38	6
9	295	5,240,000	5·9	10,250	69	0	0	28	3
10	315	3,690,000	7·1	8,020	42	2	1	49	6
11	326	4,290,000	6·5	5,200	46	0	2	48	4
12	310	5,140,000	6·4	7,460	44	1	2	45	8
Average	325·4	4,620,000	6·8	8,002	47	1·4	0·8	46	4·8

(*b*) *The Bone Marrow.*—A detailed description of the bone marrow in healthy guinea-pigs is hardly necessary at the present time. It should, however, be remembered that in the guinea-pig the marrow of the long bones is almost entirely hæmopoietic, since it contains only a very little fatty tissue. The proportion of fat to hæmopoietic tissue is seen in Plate I., Fig. 1, where the various members of the leucoblastic and erythroblastic series of cells can be seen lying in a meshwork of connective tissue fibrils and branched reticular cells. Small capillaries lined by definite endothelial cells are noticeable at certain points, while a conspicuous feature is the large number of giant cells.

Definite changes occur in the bone marrow in acute scurvy. The marrow at the diaphyses loses its normal cellular appearance: the hæmopoietic cells disappear and are gradually replaced by a more or less gelatinous material through which run a few strands of fibrous tissue. Small collections of red cells or of freshly formed blood pigment may be found scattered throughout this area which constitutes the frame-work marrow or "Geruestmark," first described by Schœdel and Nauwerk (1900 [4]). When fractures occur near the extremities of the long bones, or when the epiphysis becomes separated from the diaphysis, there is often a very considerable formation of rather dense fibrous tissue. In the more central part of the marrow the most noticeable feature is the extreme degree of congestion (Plate I., Fig. 2): the blood sinuses are closely packed with fully-formed erythrocytes, while outside the actual sinuses are found masses of red cells either of quite normal aspect or apparently in process of breaking down into granular pigment. In certain areas there seems to be an increased formation of normoblasts, but on the whole the erythroblastic reaction is very

slight. In a few cases there is a feeble myelocytic reaction, though here again the response is much less marked than in cases of simple hæmorrhage, probably owing to the intense congestion and hæmorrhage into the marrow.

Degenerative changes were noticeable in the hæmopoietic cells situated in the frame-work marrow, but in the shafts of the long bones there was no actual degeneration either in the leucoblastic cells or in the megakaryocytes. This latter fact is of interest in connection with the failure to demonstrate a diminution in the number of blood platelets in scurvy.

In chronic scurvy the bone marrow does not present the same congested appearance as in the acute form. The frame-work marrow is still present, but instead of being restricted to a narrow layer in close relation to the diaphysis, there is a tendency for areas of gelatinous degeneration to be found replacing the hæmopoietic tissue throughout the marrow (Plate I., Figs. 3 and 4). These areas of gelatinous degeneration consist of a homogeneous interstitial ground substance in which are found a few fibrous tissue elements together, in many areas, with a certain amount of blood pigment. This blood pigment may possibly indicate that these gelatinous areas are formed at the site of hæmorrhages of some standing. As a rule, the gelatinous degeneration is more marked the longer the animal has survived on a scurvy-producing diet. In a few instances there was noted in the marrow somewhat extensive areas of hyaline change in which the ground substance appeared entirely homogeneous, while the number, both of leucoblastic and erythroblastic cells, was greatly reduced. It seems possible that this hyaline change is merely a later stage of the gelatinous degeneration when the fibrous reticulum, which even in the normal guinea-pig is very delicate, has entirely disappeared. The occurrence of these changes in the bone marrow is somewhat akin to the fibrosis which was described by Zilva and Wells (1919[5]) in the tooth pulp in scurvy. Apart from the reduction in numbers, there was no definite retrogressive change in the hæmopoietic cells.

(*c*) *The Lymphoid Tissue.*—Little need be said in regard to the appearances of the lymphoid tissue in scurvy. In the acute stage the spleen usually shows extreme congestion. The splenic sinuses are packed with red cells, many of which are being actively phagocyted by the endothelial cells. Occasionally small hæmorrhages may be found in the spleen, more especially beneath the capsule. In the chronic stage there is far less congestion in the spleen; active phagocytosis still continues while a good deal of blood pigment, for the most part hæmosiderin, is found both intra- and extra-cellularly. There is no atrophy of the spleen either in the acute or chronic form of scurvy. Atrophy of the lymph glands, thymus and lymphatic tissue of the intestine is also absent. Hæmorrhages into the lymph glands and thymus are practically never seen in scurvy.

III. Bacterial Infection in Scurvy.

The belief that scurvy was a specific bacterial infection was quite commonly held until within a few years ago, more especially in Russia. Since it has been conclusively shown that scurvy can be produced at will in the guinea-pig by the removal of a definite factor from the diet, the infective theory of scurvy has been almost entirely abandoned: the more so as the universal experience of all those who have kept animals on deficient diets has shown that they readily succumb to bacterial infections while control animals on complete diets do not die from these infections.

Despite the frequency of epidemics of infectious disease among small laboratory animals, it was only comparatively recently that the relationship of these epidemics to inadequate diet was demonstrated by Theobald Smith (1913[6]). This observer found that epidemics of pneumonia due to the pneumococcus and bacillus bronchisepticus were very common in guinea-pigs during the winter months when the diet was deficient in green food, while in summer, when excess of fresh food was provided, no cases of pneumonia occurred.

Up to the present comparatively few attempts have been made to determine the cause of this liability to terminal bacterial infections. Hektoen (1914[7]) observed a normal formation of antibodies on artificial diet, while later Zilva (1919[8]) examined the formation of agglutinins, immune body and complement in rats and guinea-pigs fed on diets poor in vitamins. He could determine no diminution in the power to produce these substances. More recently Findlay and Mackenzie (1922[9]) have studied the opsonic activity of the serum in animals on deficient diets without, however, finding any definite change.

In so far as the serum reactions are concerned, there is thus little evidence of a rupture in the defence mechanism of the body as the result of a diet deficient in vitamin C. Attention was therefore directed to the cellular reaction to infection in scorbutic animals, especially as histological studies had shown that there were definite lesions in the bone marrow as the result of a deficiency of the anti-scorbutic factor. For this investigation guinea-pigs with chronic scurvy were alone employed. Experiments were carried out on control animals and on those with chronic scurvy with the following organisms, viz., *Pneumococcus, Staphylococcus aureus, Streptococcus hæmolyticus* and *B. coli.* The two points to which special attention was directed are:—

(1) The number of organisms and the time required to produce a fatal result.

(2) The cellular reaction to the infection as shown by changes in the blood and hæmopoietic organs.

Technique.

The animals employed for these experiments were all placed on the standard diet of oats and bran *ad libitum* with the addition of a daily ration of 70 c c. of autoclaved milk. In the case of the scorbutic animals 2 c.c. of orange juice were given every third day, while the control animals received a daily ration of swedish turnips. The scorbutic animals were allowed to pass six weeks on the diet before they were infected with a suspension of the particular

organism under investigation. In the case of the pneumococcus two sets of controls were employed—(1) animals of the same weight at the beginning of the experiment, which, as the result of a full diet, had grown normally, and (2) animals of the same weight as the scorbutic ones at the time of injection. As a rule, therefore, the scorbutic animals were older than the controls in the second group, a point which may possibly be of some importance, since Südmersen and Glenny (1909 [10]) have shown that the susceptibility to diphtheria toxin

TABLE II.

The Effects of Intraperitoneal Injection of Pneumococcus *in Guinea-Pigs.*

No. of Animal.	Condition.	Weight in Grams.	No. of Pneumo-cocci in millions.	Period of Survival in days.	Remarks.
1	Scorbutic .	320	500	...	Killed after 15 days; no peritonitis.
2	,, .	347	500	12	Peritonitis. P. from exudate and heart blood.
3	,, .	310	500	...	...
4	,, .	330	500	...	...
5	Non-scorbutic .	315	500	...	Killed after 15 days; no peritonitis.
6	,, .	322	500	...	...
7	,, .	340	500	8	Peritonitis. P. from exudate and heart blood.
8	,, .	312	500	10	Do. do.
9	,, .	406	500	...	Killed after 15 days; no peritonitis.
10	,, .	415	500	...	...
11	,, .	430	500	...	...
12	,, .	424	500	...	...
13	Scorbutic .	304	1000	2	Slight peritoneal exudate. P. from heart blood.
14	,, .	345	1000	1·5	Do. do.
15	,, .	362	1000	4	Do. do.
16	,, .	320	1000	3	Do. do.
17	Non-scorbutic .	315	1000	6	Peritonitis. P. from heart blood.
18	,, .	310	1000	...	Killed after 15 days; no peritonitis.
19	,, .	342	1000	12	Peritonitis. P. from heart blood.
20	,, .	336	1000	8	Do. do.
21	,, .	420	1000	10	Do. do.
22	,, .	368	1000	8	Do. do.
23	,, .	390	1000	9	Do. do.
24	,, .	348	1000	6	Do. do.
25	Scorbutic .	295	2000	2	Slight peritoneal exudate. P. from heart blood.
26	,, .	315	2000	3	Do. do.
27	,, .	328	2000	2	Do. do.
28	,, .	310	2000	2	Do. do.
29	Non-scorbutic .	265	2000	4	Slight peritonitis. P. from heart blood.
30	,, .	322	2000	6	Peritonitis. P. from heart blood.
31	,, .	345	2000	8	Do. do.
32	,, .	280	2000	6	Do. do.
33	,, .	364	2000	6	Do. do.
34	,, .	385	2000	6	Do. do.
35	,, .	391	2000	4	Do. do.
36	,, .	412	2000	7	Do. do.

of guinea-pigs of constant weight varies inversely as their age. All the inoculation experiments were carried out during the winter of 1921-22. The organisms for injection were in each case grown on a suitable solid medium for twenty-four hours: they were then washed off with normal saline and a suitable dose was calculated by the opacity method by comparison with suspensions of barium sulphate.

(a) Pneumococcus.

As is well known, the susceptibility of different species of animals to infection with the pneumococcus varies very considerably. Mice are the most susceptible: then in order of decreasing susceptibility come rabbits, rats, sheep, guinea-pigs and dogs. Pigeons are said to be immune. As a rule, the more susceptible animals die from a pneumococcal septicæmia, while in the less susceptible species the local lesion is of greater severity than the general reaction.

The strain of pneumococcus used in the present experiments belonged to type 2. It was originally isolated from a case of pneumococcal meningitis, but had been grown for some weeks on artificial media. No attempt was made to increase its virulence by animal passage.

Experiment I.

In this experiment twelve guinea-pigs suffering from chronic scurvy were inoculated intraperitoneally with from 500 to 2000 million pneumococci. Twelve normal guinea-pigs of the same age as the scorbutic but of heavier weight and twelve younger guinea-pigs of the same weight were also inoculated intraperitoneally. The times of survival and other data are shown in Table II.

Experiment II.

Twelve guinea-pigs, four of them with chronic scurvy and eight controls, were inoculated under the skin of the abdomen. The controls, as in Experiment I., were divided into two groups according to age and weight. (See Table III.)

TABLE III.

The Effects of Subcutaneous Injection of Pneumococcus *in Guinea-Pigs.*

No. of Animal.	Condition.	Weight in Grams.	No. of Pneumococci in millions.	Period of Survival in days.	Remarks.
37	Scorbutic .	246	1000	8	P. from local lesion and heart blood.
38	,, .	260	1000	8	Do. do.
39	,, .	255	1000	6	Do. do.
40	,, .	282	1000	5	Do. do.
41	Non-scorbutic .	265	1000	...	...
42	,, .	284	1000	...	...
43	,, .	250	1000	8	P. from local lesion and heart blood.
44	,, .	224	1000	6	Do. do.
45	,, .	304	1000	...	Killed after 15 days; no local lesion.
46	,, .	320	1000	...	...
47	,, .	347	1000	8	Local lesion. P. from lesion.
48	,, .	340	1000	...	...

A study was also made of the changes in the leucocyte count following the intraperitoneal injection of pneumococci. Both in the scorbutic and control animals there was a marked leucopenia immediately following the injection. This persisted for about six hours in the animals that survived for more than three days, and was then followed by a leucocytosis. In those cases where the animal died from pneumococcal septicæmia in a few hours the leucopenia persisted up till death, while in the case of animals such as guinea-pig No. 21 which died of an extensive pneumococcal peritonitis after nine days, the leucocytosis was followed by a second leucopenia. Leucocyte counts of typical animals are seen in Table IV.

TABLE IV.

Leucocyte Changes following Injection of Pneumococcus *in Guinea-Pigs.*

No. of Animal.	Condition.	Before Injection.	3 hrs. after	1 day.	2 days.	3 days.	4 days.	5 days.	6 days.	7 days.	8 days.	9 days.
1	Scorbutic . .	9,340	5420	25,810	26,900	23,430	20,625	19,840	16,250	11,100	12,240	Survived.
21	Non-scorbutic .	8,670	5204	18,730	16,330	12,000	10,700	6,440	7,280	5,420	4,110	Died.
13	Scorbutic . .	9,840	6470	5,810	3,430	Died	..	..	..	..	..	..
5	Non-scorbutic .	10,930	6220	11,800	22,500	21,220	25,450	15,400	12,320	10,250	10,480	Survived.

There thus appear to be two forms of leucopenia—a primary form which occurs immediately after the injection of organisms whether this injection be subcutaneous, intravenous, or intraperitoneal, and a secondary form which occurs at a late stage of the disease and usually portends a fatal result. The primary form appears to be due to a rearrangement of the leucocytes in the circulation, while the secondary form is indicative of a failure on the part of the bone marrow to react to infection. In the case of intravenous and intraperitoneal injections of organisms, it is of importance to remember that bacteria reach the bone marrow a very short time after their injection. If these organisms persist in the marrow their presence naturally tends to interfere with the occurrence of a leucocytosis in the peripheral blood stream. These experiments show that with small doses of pneumococci—500 million—guinea-pigs, both healthy and scorbutic, show little or no difference in their powers of resistance, since death only took place in one out of four scorbutic animals and in two out of eight control animals. Guinea-pigs No. 1 (scorbutic), Nos. 5 and 9 (non-scorbutic) which were killed fifteen days after the injection showed no signs of peritonitis. Cultures from the surface of the peritoneum were in every case sterile, while smears examined microscopically showed only a few mononuclear cells in various stages of degeneration. More striking results were obtained with animals injected intraperitoneally with 1000 million pneumococci. The four scorbutic animals only

survived for an average period of 2·6 days, while of the non-scorbutic guinea-pigs one survived while the other eight died in an average of 8·4 days. In the scorbutic animals the exudate into the peritoneal cavity consisted only of a little serous fluid, while in the control animals there were dense yellowish flakes of fibrin on the peritoneal surface. In the scorbutic animals death was thus essentially due to a pneumococcal septicæmia, very similar to that which occurs in the mouse, while in the control animals death was associated with the presence of a well-marked peritonitis.

A study of the differential leucocyte counts was also of some interest in this connection. (See Table V.).

TABLE V.

Differential Leucocyte Counts in Scorbutic and Control Guinea-Pigs Inoculated Intraperitoneally with 100 *million* Pneumococci.

Date.	Polymorph-leutrophil.	Eosinophil.	Basophil.	Mononuclear.	Lymphocyte.	Myelocyte.	Dead.
GUINEA-PIG 16—SCORBUTIC.							
Before injection .	42	4	1	7	46	...	...
3 hours after injection .	48	1	2	4	45	...	...
1 day ,, .	55	1	3	8	32	...	...
2 days ,, .	56	4	2	9	27	...	2
3 ,, ,, . (animal moribund)	32	1	3	6	55	2	4
GUINEA-PIG 20—NON-SCORBUTIC.							
Before injection . .	47	2	0	8	43	...	...
3 hours after injection .	41	2	0	5	54	...	...
1 day ,, .	57	1	2	6	34	...	...
2 days ,, .	58	2	3	7	30	...	...
3 ,, ,, .	51	1	1	4	43	...	...
4 ,, ,, .	49	1	2	8	40	...	...
5 ,, ,, .	54	1	3	5	35	...	2
6 ,, ,, .	40	2	2	6	48	...	2
7 ,, ,, .	36	1	2	7	47	3	4

In the scorbutic guinea-pig No. 16, degenerative changes in the leucocytes were met with on the second day after inoculation, while in the control No. 20 they did not appear till the fifth day after inoculation. These degenerative changes in the polymorphs consist of a swelling-up and disintegration of the nuclei while the protoplasm becomes vacuolated and the granules lose their characteristic staining reactions. The appearance of myelocytes immediately before death is of interest since Tongs (1921 [11]), as the result of a recent study of the effects of hæmolytic streptococci on the blood and hæmopoietic