VITAMINS AND BACTERIAL INFECTION

by

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INTORDUCTION

Despite the research that has already been carried out on the subject of immunity there still remains much to be explained in regard to the methods by which multicellular organisms guard themselves against the attacks of pathogenic bacteria. In the first place, as Bordet (1920) has pointed out, the actual penetration of the integument is no easy matter: the hardness of the skin, the activity of ciliated epithelium, the secretion of acid in the stomach, the peristalsis of the intestine all help to protect the animal. When once bacteria have penetrated the tissues, two other mechanisms of defence come into play in the phagocytic activities of certain cells and the bacterial powers of the serum. Both phagocytosis and the immune reactions of the serum have received considerable study, but at present the factors which influence the penetrability of the skin and mucous membranes are pratically unknown. The investigation of these factors/

factors from the experimental point of view is obviously a matter of considerable difficulty: it may quite well be that bacteria are constantly penetrating the tissues in small numbers, only to be destroyed by the phagocytes of the body. Such invasions being on so slight a scale would give rise to no local or general symptoms and would thus pass unheeded.

From the clinical point of view it has long been recognised that the natural immunity of a particular animal to a particular micro-organism might be influenced by such factors as an insufficiency of food, exposure to cold or another bacterial infection.

The investigations herein described form part of an extensive enquiry, which is at present being undertaken, on the relationship of diet to immunity. The following experiments deal more particularly with the relationship of bacterial infection to diets deficient in those little known substances, the vitamins. The experiments were all carried out in the Research Laboratory of the Royal College of Physicians' of Edinburgh, to whom and to Col. McKendrick, Superintendent of the Laboratory, my sincere thanks are due. For the investigation of vitamin C, the experimental animals used were guinea-pigs; in the case of vitamin B, rats and pigeons were employed, while in the case of vitamin A, rats alone were used. The investigations have consisted partly of a detailed examination of the blood and haemopoietic organs of animals suffering from deficiency/

deficiency diseases, partly of a bacteriological examination of the actual degree of resistance possessed by such animals.

1. DEFICIENCY IN VITAMIN C. and BACTERIAL INFECTION.

(a) Experimental Scurvy in the Guinea-Pig.

Theobald Smith (1895[2]) was the first to show that guinea-pigs, when fed on a diet of oats and bran, developed a disease characterised by extensive haemorrhages. The relationship of this haemorrhagic condition to scurvy was, however, only established definitely by Holst and Frolich (1907[3]). The original diet used by these observers consisted of oats, bran and hay. Chick and Hume (1917[4]) however found that the addition of a daily ration of 70 c.cs of milk to this diet improved the condition of the experimental animals, although it did not delay the onset of the scorbutic symptoms. They believed that the improved nutrition of the animals was due to the vitamin A contained in the milk. Some doubt, however, has recently been thrown on this conception by the fact that as Stammers (1921[5]) points out bran contains a considerable amount of vitamin A. Another possible explanation of the improved nourishment after the addition of the milk might of course be the increased salt ration. In order to determine this point the following experiment was carried/

carried out:-

Four young guinea-pigs varying in weight from 269 to 328 grs. were placed on an ad libitum diet of oats and bran with the daily addition of 5 c.cs of orange juice and 70 c.cs of autoclaved milk. Four other guinea-pigs of about the same weight were placed on a similar diet without the ration of 70 c.cs of milk, but with the addition of 0.7 grs. a day of the following salt mixture -

Salt mixture corresponding to the salts in 100 grams of Cow's milk.

NaCE.....	10.62.	
KCE.	9.16.	
KH_2PO_4.....	12.77.	
K_2HPO_4.....	9.22.	
K_3Citrate....	5.47.	
$MgHPO_4$	3.71.	
MgCitrate....	4.05.	
$CaHPO_4$.....	7.42.	
$Ca_3(PO_4)_2$....	8.90.	
Ca citrate...	23,55.	
$CaCo_3$.....	10.0	(to represent lime combined with protein).

The average weight curves of the two groups of animals are shown in chart 1, from which it will be seen that the growth of the animals on the salt mixture is only slightly inferior to that of the animals receiving the milk ration. There is, however, a difference in favour of the latter group of animals so that doubtless the high ration of vitamin A does to a certain extent improve the nutrition. On the other hand vitamin A

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CHART I

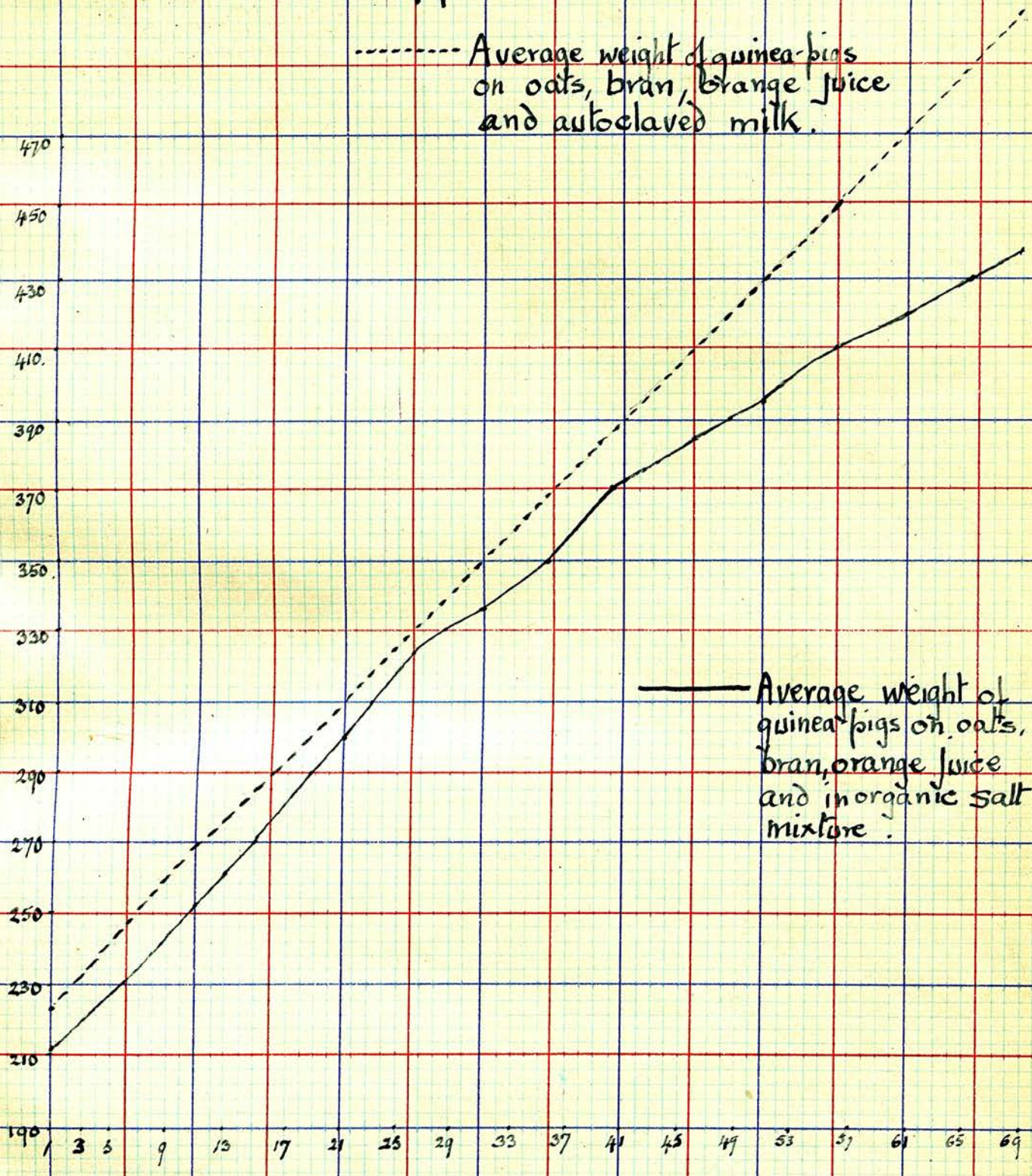
Average weight of guinea-pigs on oats, bran, orange juice and autoclaved milk.
Average weight of guinea-pigs on oats, bran, orange juice and inorganic salt mixture.
470
450
430
410
390
370
350
330
310
290
270
250
230
210
190
1
3
5
9
13
17
21
25
29
33
37
41
45
49
53
57
61
65
69

CHART II

Average Weight Curve

of

Six guinea-pigs placed on diet of extracted oatmeal and bran, salt mixture and orange juice. (average life – 39 days).

is necessary for the adequate growth of guinea-pigs as is shown by the following experiments.

Six guinea-pigs varying in weight from 259 grs. to 386 grs. were placed on a diet of oatmeal and bran, which had been extracted first with ether, then with absolute alcohol and finally autoclaved at 120°C for twenty-four hours. In addition each animal received a daily ration of 5 c.cs of orange juice and 0.7 grs. of the inorganic salt mixture. The weight curves are shown in chart 11: it will be seen that there was practically no growth, while death occurred in an average of 38 days. For all practical purposes the diet of oats, bran and autoclaved milk may therefore be looked upon as complete in every way except as regards the absence of vitamin C. On microscopical examination of the costo-chondral junctions, there was marked osteo-porosis of the bones with reduction in the length of the rows of cartilage cells. These experiments tend to confirm the findings described by Tozer (1921[6]) as occurring in guinea-pigs fed on a diet deficient in vitamin A. When placed on such a diet of oats, bran & milk guinea-pigs at first show no symptoms. About the twelfth to fifteenth day, however, there is usually some tenderness at the knee joints, which in a short time become definitely swollen. There is also not infrequently pain in the masseter muscles, when the animal may assume what has been aptly termed the face-

ache/

ache position. In other cases the animal lies with one hind leg fully extended. A day or two before death the coat becomes ragged and staring, while a terminal diarrhoea is not uncommon. Finally the animal passes into a semi-comatose condition which eventually ends in death. This usually takes place from the twenty-fifth to the thirty-fifth day, though some animals manage to survive for a somewhat longer period. For the first fortnight there is usually no loss of body weight, in fact in not a few animals a slight increase in weight may occur. During the last two or three days of life, however, a definite loss of weight does occur, probably because during this period the animal eats little or no food. The loss of weight at death generally amounts to about a third of the original body weight. Bacterial infection is not very common in guinea-pigs with acute scurvy. In twenty-four animals suffering from acute scurvy, the heart blood was sterile in all except one from which a small gram positive diplococcus was isolated.
The post mortem appearances in acute guinea-pig scurvy are very charteristic. The knee and wrist joints are definitely swollen, while recent haemorrhages into the surrounding muscles are almost always seen. Haemorrhages are also not infrequently found in the loose tissues of the axillae as well as in the intercostal and masseter muscles. Acute scurvy in the guinea-pig differs in one respect from that met

with/

with in man in that the gums do not show any gross macroscopic lesions. Occasionally there is slight hyperaemia round the bases of the two lower incisors, but the gums never assume the spongy condition so characteristic of human scurvy. This condition would appear to be largely due to a secondary bacterial infection of the gums. The costo-chondral junctions are always definitely enlarged. This enlargement is least marked in the upper and floating ribs: it is best seen on opening the thorax and examining the inner surface of the thoracic wall. Small subperiosteal haemorrhages are not infrequently found in relation to the ribs, while spontaneous fractures are very common both in the ribs and long bones. Even more common than actual fractures are separation of epiphyses, more especially the epiphysis at the upper end of the tibia. Small haemorrhages into the pericardium and pleura are occasionally met with as are petecchial haemorrhages in the peritoneum and intestine. There is no atrophy of the spleen, thymus or lymphoid tissue. The adrenals are always definitely enlarged, usually to almost double their normal size, a fact first pointed out by Rondoni and Morganini (1915[7]), while McCarrison (1919[8]) has found that their adrenalin content is usually increased. Apart from the microscopic changes in the blood and blood-forming organs lesions have been noted more especially in the teeth, bones and blood vessels/

vessels.

Zilva and Wells (1919[9]) have described marked fibroid degeneration of the tooth pulp, involving cells, nerves, blood vessels and odontoblasts with the result that no trace of cellular organisation, no trace of cell nuclei and no interstitial substance can be found. It is of especial interest to note that these changes are well marked even when other signs of scurvy are almost wanting.

The changes in the bones are especially well seen at the costo-chondral junctions, where the rows of cartilage cells are shortened or entirely disorganised, while the trabeculae are dwarfed or have almost disappeared. The changes in the bones were very accurately described by Holst and Frolich (1907[8]) in their classisal work on guinea-pig scurvy, while more recently they have been closely investigated by Delf and Tozer (1918[10]).

The changes in the capillary wall are of extreme interest. Findlay (1921[11]) found that about ten days after placing the animals on a scorbutic diet the endothelial cells became swollen and granular. This was later associated with extreme capillary congestion, with a very fine degree of oedema and finally with the passage of red blood corpuscles from the capillaries by a process of diapedesis.

In order to protect completely a guinea-pig from scurvy a daily dose of at least 5 c.cs of orange juice must be added/

added to the oats, bran, milk diet. When a smaller quantity, such as 2 c.cs, is given every third day the animal passes into a condition of chronic scurvy. From the clinical point of view the symptoms to be noted in animals with chronic scurvy are practically non-existent. Close observation, however, shows that the animals are less active than usual and in addition they fail to put on weight. Either their weight remains practically stationary, or it slowly decreases. Guinea-pigs may be maintained in this condition of chronic scurvy for many weeks at a time. In its absence of definite clinical symptoms, chronic scurvy in the guinea-pig is thus not unlike the form of latent scurvy described by Hess (1917[12]) in children in whom there are no definite clinical symptoms except failure of growth and some indefinite malaise. Eventually guinea-pigs fed on this diet deficient in vitamin C die, usually from some intercurrent bacterial infection, Hess (1921[13]) states that many of his scorbutic animals died from broncho-pneumonia. He does not, however, state the exact diet on which his animals were fed, a point of some importance since in my experience broncho-pneumonia is common only as a fatal termination in animals, guinea-pigs and rats-suffering from a lack of vitamin A in the food, either alone or in addition to a deficiency of vitamin C. Thus of the six guinea-pigs fed on the autoclaved and extracted oats and bran only one developed keratomalacia

in/

in one eye, while four showed broncho-pneumonic changes in the lung.

When uninfected guinea-pigs are killed after six weeks on a diet of oats, bran, milk and 2 c.cs of orange juice every third day, the macroscopic appearances are far from striking. There is only slight, if any, swelling of the knee and wrist joints, while the enlargement of the costo-chondral junctions is usually less marked than in the acute condition. Fractures united only by a little poorly formed callus are by no means rare, while recent fractures, possibly made while handling the body after death are very common. Macroscopic haemorrhages are very rarely seen, while the adrenal is not nearly so congested or enlarged as in the acute stage of the disease.

Microscopically the bones in many cases show some osteoporosis, while the osseous laminae are thin and reduced in number. The alterations in the intermediate cartilages are very characteristic, especially at the costo-chondral junctions. The rows of cartilage cells are much shortened, but do not as a rule show much distortion, while an ossified band extends across the junction. Delf and Tozer (1918)[10] regard this ossified bar as an attempt to strengthen the junction in an abnormal manner.

In the vascular system the congestion of the smaller venules and capillaries is not as marked a feature as in acute scurvy. Examination of the capillary endothelium/

endothelium shows that at many points the cells are swollen and laden with haemosiderin granules. A few recent haemorrhagic foci can occasionally be found in the lungs,

(b) The Blood and Haemopoietic organs in Guinea-Pig Scurvy.

(1) The Blood. Numerous investigators have at various times examined the blood in normal guinea-pigs. A summary of the main findings in the blood is given in Table I -

TABLE I.

BLOOD CELLS IN NORMAL GUINEA-PIGS.

OBSERVER	Goodall (1910[14])	Bedson [15&16] (1921-22)	Burnett (1908[17])	Ehrlich (1910[18])	Author
Red cells average.	5,600,000.	4,240,000 - 6,496,000. 5,460,000.	5,500,000.	.....	4,600,000 - 6,240,000 5300,000.
Blood platelets average.	.....	588,000 - 1,160,000 797,000.	.....	.....	592,000 - 987,000 745,000.
Leucocytes average.	9,170.	4,600 - 8,400. 6,800.	9,000.	.....	7250 - 12,460. 9,4000.
Polymorphonuclears.	37.	50.	47.	45.	44.
Eosinophils	3.	2.	2	4	3
Basophils	-	1.	1	-	1
Lymphocytes	60.	40	50.	33·5	43
Mononuclears		7.		17·5	9.

In the guinea-pig, as in many other small rodents the lymphocyte series of cells is more numerous than in man. The polymorphonuclear leucocytes also instead of having neutrophil granules have numerous small granular masses, which with modifications of the Romanowsky stain take on a definite pink colour. These polymorphonuclear cells are, however, quite distinct from the coarsely granular eosinophils.

They/

They are functionally identical with the neutrophils in man. Goodall (1910[14]) has shown that there is a definite relation between the number of leucocytes and the weight of the animal, the heavier the guinea-pig the greater the number of leucocytes. In certain of the mononuclear cells cell-inclusions, known as Kurloff bodies are not infrequently seen. The significance of these bodies is quite unknown, though Woodcock (1921[19]) has recently suggested that they are the remains of phagocyted red cells which have been incompletely digested.

In acute scurvy there is little or no reduction in the number of red cells in the blood; in fact if blood from the peripheral vessels alone be examined there may in some instances be an apparent increase in the red cell count - a result due in all probability to the capillary stasis, which may occur in acute scurvy. Bedson (1921[15]) found that the number of red cells varied from 4,240,000 to 6,496,000 per c.mm while Findlay (1921[11]) noted a variation of from 4,600,000 to 5,120,000 per c.mm, the average being 4,950,000 - a figure practically identical with the average count from controls. A certain number of the erythrocytes showed polychromatophilia and poikilocytosis, while occasionally normoblasts were noted in the peripheral circulation.

Bedson (1921[15]) could not determine any diminution in the number of the blood platelets, a result of considerable interest in view of their relationship to the purpuric condition/

condition.

No characteristic change could be noted either in the total number or in the differential count of the leucocytes. In a few instances there was a definite leucocytosis, which was possibly produced by the haemorrhage, accompanied by the presence of lymphoidocyte like cells in the peripheral blood stream. These primitive cells were most noticeable in one case where the leucocytosis amounted to 22,000 per c.mm. 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 are shown in Table II.

TABLE II.

BLOOD CHANGES IN GUINEA-PIGS WITH CHRONIC SCURVY.

No. of animal	Weight in grams.	No. of Red cells per c.mm.	Ratio of platelets to red cells	No. of leucocytes per c.mm.	Polymorphonuclear leucocytes	Eosinophils	Basophils.	Lymphocytes.	Mononuclears.
1.	340.	5,180,000.	1 : 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.	8180.	42.	4	1	46.	7
6.	345.	4,600,000	7·3.	6540.	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.	1 : 6·8.	8,002.	47.	1·4.	0·8.	46.	4·8

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 4,600,000 per c.mm. Polychromatophilia and nucleated reds were less common than in the acute stage. Estimations of the blood platelets, using the method described by Bedson (1922[4]) 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[20]) there is usually a relative lymphocytosis.

11. 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 haemopoietic, since it contains only a very little fatty tissue. The proportion of fat to haemo-poietic tissue is seen in 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/

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 disphyses loses its normal cellular appearance; the haemopoietic 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 Schoedel and Nauwerk (1900)[21]. 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 (see 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 slight myelocytic reaction, though

here/a

here again the response is much less marked than in cases of simple haemorrhage, probably owing to the intense congestion and haemorrhage into the marrow. Carnegie Dickson (1908)[22] points out that in acute septicaemias the occurence of haemorrhage into the bone marrow is one of the most important factors leading to an imperfect reaction on the part of the leucoblastic cells.

Degenerative changes were noticeable in the haemopoietic 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 haemopoietic tissue through the marrow. 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, which in all probability/

probability indicates that these gelatinous areas are formed at the site of haemorrhages 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. This appearance is indicated in Fig. 111. It seems possible that this hyaline change is merely a later stage of the gelatinous degeneration in which 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) in the tooth pulp in scurvy. Apart from the reduction in numbers there was no definite retrogressive change in the haemopoietic cells

111. 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 phagocytosed by the endothelial cells. Occasionally small haemorrhages 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/

phagocytosis still continues, while a good deal of blood pigment, for the most part haemosiderin, 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 conspicuous by its absence. This is of interest as showing that there is little real connection between the pathology of deficiencies due to vitamin C and those due to vitamin B. When animals are fed on diets deficient in vitamin B there is always noted a marked atrophy of the lymphoid tissues throughout the body together with a reduction in the number of the lymphocytes in the peripheral blood stream. Haemorrhages into the lymph glands and thymus are practically never seen in scurvy.

SUMMARY.

When vitamin C is entirely removed from the guinea-pig's diet the animal dies in a few weeks from acute scurvy. When vitamin C is given in subminimal amounts the animal ceases to grow and after a considerable period dies, usually as the result of an intercurrent infection. After death, the pathological changes characteristic of chronic scurvy can be noted. There are no definite blood changes either in acute or chronic scurvy. In the haemopoietic organs there is no atrophy of the lymphoid tissue. The bone-marrow in the/

the acute stage shows haemorrhagic congestion, in the chronic stage gelatinous and hyaline degeneration.

(b) Bacterial Infection in Scurvy.

The belief that scurvy was a specific bacterial infection was quite commonly held until with a few years ago, more especially in Russia. This theory was based on the well known association of scurvy with plague, pestilence and famine and on the frequency with which organisms can be isolated from the blood of patients suffering from scurvy. In the case of experimental scurvy there have not been wanting those who like Jackson and Moore (1916)[23] have upheld the infective theory on the grounds that they have been able to isolate a small diplococcus from the tissue of scorbutic guinea-pigs. On inoculating this organism into rabbits living under ordinary conditions, Jackson and Moody (1916)[24] produced haemorrhagic and other lesions in the bones, joints, gums and muscles, which they considered to be similar to scurvy. Some doubt, however, has been thrown on this work since adult rabbits do not develop scurvy even when fed for months on a diet deficient in vitamin C. 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 bacterial theory has for the most part been abandoned, the more so as the universal experience of all those who have kept animals on deficient diets has/

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 infections 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)[25]. 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)[26] observed a normal formation of antibodies on artificial diet while later Zilva (1919)[27] 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.

In addition to the deficiency diseases, numerous other conditions such as the anaemias and especially chronic bacterial infections render the tissues more liable to the attacks of relatively non-virulent organisms. In fact so common are these terminal infections that as Osler/

Osler has expressed it "persons rarely die of the diseases with which they suffer". Some years ago Flexner (1896[28]) studied the question of terminal infections with staphylococci in human beings suffering from chronic bacterial infection. He thought that, at least in a few cases, he could detect a slight decrease in the bactericidal actions of the human serum against staphylococci. The accuracy of these findings was however rendered doubtful by Windsor and Wright (1902[29]) who showed that human blood serum has practically no bactericidal action against staphylococci. Tunnicliff (1912[30]) investigated the opsonic index during the leucopenia of measles. He found that there was a slight decrease in the index for streptococci, staphylococci and tubercle bacilli. Bartlett and Ozaki (1917[31]) injected a dog with a massive dose of bacillus coli and five hours later with a quantity of staphylococci. There was no decrease in the opsonic index for staphylococcus. Quite recently Cross (1921[32]) has investigated the question of the opsonic index in relation to terminal infections. He was unable to determine any decrease in activity against any bacteria not concerned in the primary infection even in the very last stages of fatal infection. In those cases where the natural immunity is lowered by a primary bacterial infection there is thus little evidence of any loss in opsonic activity. As the opsonic activity of the serum in animals/

animals fed on deficient diets does not appear to have been investigated, experiments were carried out to determine whether there was any reduction in the opsonic activity as the result of a diet deficient in vitamin C.

Technique.

Four guinea-pigs were placed on an ad libitum diet of oats, bran and 70 c.cs of autoclaved milk per diem with with the addition of 2 c.cs of orange juice every third day. Four controls were placed on the same basal diet but with the addition of 10 c.cs of orange juice every day. Estimations were carried out with two organisms bacillus coli and staphylococcus aureus. Readings were made at the beginning of the experiment and at weekly intervals for the next six weeks. 0.5 c.cs of blood was removed from the heart of each animal, the sera from the controls and the scorbutics being each pooled before use. The opsonic technique used was essentially that recommended by Wright. One hundred polymorphonuclear leucocytes were examined on each slide and notes were taken of the number of leucocytes containing bacteria and of the total number of bacteria phagocytosed. The average number of bacteria per polymorphonuclear leucocyte has been termed the phagocytic index. The results are summarised in Table 111.

TABLE III.

Opsonic activity in healthy and Scorbutic guinea-pigs.

Date.	Organism.	Condition of Animal.	Number of polymorphs counted.	Number of cells with bacteria.	Total number of organisms ingested.	Phagocytic index.
5.2.22	Staphylococcus.	Scorbutic.	100.	96.	306.	3·0.
		control.	100.	94	312.	3·1.
	B. coli.	Scorbutic	100.	90	186.	1·9.
		control.	100.	86.	205.	2·0.
12.2.22	Staphylococcus.	Scorbutic	100.	84	352.	3·5.
		control.	100.	92	296.	3·0.
	B. coli.	Scorbutic	100.	91	106.	1·1.
		control.	100.	87.	192.	1·9.
19.2.22	Staphylococcus.	Scorbutic.	100.	90.	311.	3·1.
		control.	100.	88	334.	3·3.
	B. coli.	Scorbutic	100.	94.	111.	1·1.
		control	100.	84.	146.	1·5.
26.2.22	Staphylococcus	Scorbutic	100.	88.	296.	3·0.
		control	100.	95.	290.	2·9.
	B. coli.	Scorbutic	100.	87.	71.	0·7.
		control.	100.	92.	84.	0·8.
5.3.22.	Staphylococcus	Scorbutic	100.	94.	315.	3·1.
		control.	100.	91.	330.	3·3.
	B. coli.	Scorbutic	100.	87.	115.	1·1.
		control.	100.	84.	118.	1·2.
12.3.22.	Staphylococcus.	Scorbutic	100.	93.	327	3·3.
		Control.	100.	91.	319.	3·2.
	B. coli.	Scorbutic	100.	84.	65.	6·5.
		control.	100.	94.	76.	7·6.
19.3.22.	Staphylococcus.	Scorbutic	100.	87.	220.	2·2
		Control.	100	96.	204.	2·0.
	B. coli.	Scorbutic	100	89.	75	0·7
		control	100	92.	81.	0·8

These observations though they require extending over a larger series of animals and a greater number of bacterial species nevertheless do not indicate any reduction in the opsonic power of the serum as the result of a diet deficient in vitamin C. They thus tend still further to strengthen the evidence in support of the view first expressed by Bordet (1909)[33] that the phagocytic activity of the body is a relatively stable function and one not easily influenced by conditions which profoundly affect other vital activities.

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 previously 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. When animals are suffering from acute scurvy their hold on life is in any case so precarious that it is almost impossible to determine whether their death is due to the bacterial infection or to the scurvy. To guinea-pigs suffering from chronic scurvy this objection does not apply. Experiments were therefore carried/

carried out on control animals and on those with chronic scurvy with the following organisms, viz. pneumococcus, staphylococcus, aureus, bacillus coli and streptococcus haemolyticus. 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 haemopoietic 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.cs of autoclaved milk. In the case of the scorbutic animals 2 c.cs of orange juice were given every third day, while the control animals received 10 c.cs per day. The scorbutic animals were allowed to pass six weeks on the diet before they were injected 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/

second group, a point which may possibly be of some importance since Südmersen and Glenny (1909) have shown that the susceptibility to diptheria toxin 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 septicaemia 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 II. 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/

animal passage.

Experiment 1.

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 animals 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 IV.

TABLE IV.

The effects of intraperitoneal injections of pneumococci in guinea-pigs

Number of Animal.	Condition of Animal.	Weight in grams.	No. of pneumococci in millions.	Period of survival in days.	Remarks.
1.	Scorbutic	320.	500.	–	Killed after 15 days: no peritonitis.
2.	"	347.	500.	12.	Peritonitis: pneumococcus from 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: pneumococcus from heart blood.
8.	"	312.	500.	10.	" " " "
9.	Non-Scorbutic	406.	500.	–	Killed after 15 days no peritonitis
10.	"	415.	500.	–	" " " "
11.	"	430.	500.	–	" " " "
12.	"	424.	500	–	" " " "
13.	Scorbutic	304.	1000.	2.	slight peritonitis: pneumococcus from heart blood.
14.	"	345.	1000.	1·5	" " " "
15.	"	362.	1000.	4	" " " "
16.	"	320.	1000.	3.	" " " "

TABLE IV. ctd.

The effects of intraperitoneal injections of pneumococci in guinea-pigs.

Number of Animal.	Condition of Animal.	Weight in grams.	No. of pneumococci in millions	Period of survival in days.	Remarks.
17.	Non-Scorbutic	315	1000.	6	peritonitis: pneumococcus from heart blood
18.	"	310.	1000.	–	killed after 15 days: no peritonitis.
19.	"	342.	1000	12.	peritonitis: pneumococcus from heart blood
20.	"	336.	1000.	8	" " "
21.	Non-scorbutic	420.	1000.	10	peritonitis: pneumococcus from heart blood
22.	"	368.	1000.	8	" " "
23.	"	390.	1000.	9	" " "
24.	"	348.	1000	6.	" " "
25.	Scorbutic	295.	2000.	2	slight peritonitis: pneumococci from heart blood
26.	"	315.	2000.	3	" " "
27.	"	328.	2000.	2	" " "
28.	"	310.	2000.	2	" " "
29.	Non-scorbutic	265.	2000.	4	peritonitis: pneumococcus from heart blood
30.	"	322.	2000.	6.	" " "
31.	"	345.	2000.	8.	" " "
32.	"	280.	2000.	6.	" " "
33.	Non-scorbutic	364.	2000.	6.	peritonitis: pneumococcus from heart blood
34	"	385.	2000	6.	" " "
35.	"	391.	2000	4.	" " "
36.	"	412.	2000.	7.	" " "

Experiment 11.

Twelve guinea-pigs, four of them with chronic scurvy and eight controls were inoclulated under the skin of the abdomen. The controls as in experiment 1 were divided into two groups according to age and weight.

TABLE V

The effects of subcutaneous injection of pneumococci in guinea-pigs.

Number of Animal.	Condition of Animal.	Weight in grams.	Number of pneumococci in millions	Period of survival in days.	Remarks.
37.	Scorbutic	246.	1000	8	Pneumococcus from local lesion a heart blood.
38	"	260.	1000.	8	" " "
39	"	255.	1000	6	" " "
40	"	282.	1000.	5.	" " "
41.	Non-scorbutic	265.	1000.	—	
42	"	284.	1000.	—	
43	"	250.	1000.	8	Pneumococcus from local lesion a heart blood
44	"	224.	1000.	6.	" " "
45	Non-scorbutic	304.	1000.	—	killed after 15 days: no local lesion
46.	"	320.	1000	—	
47.	"	347.	1000.	8	Pneumococcus from local lesion
48	"	340.	1000.	—	

A study was also made of the changes in the leucocyte count following the intraperitoneal injection of penumococci. 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 septicaemia in a few hours the leucopenia persisted up till death, while in the case of animals, such as guinea-pig 21, which died of an extensive penumococcal peritonitis after nine days, the leucocytosis was followed by a second leucopenia. Leucocyte counts of typical animals are seen in Table Vl.

TABLE VI.

No. of animal	Condition of animal	Before injection	3 hours after.	1 day.	2 days	3 days.	4 days.	5 days	6 days.	7 days.	8 days	9 days.
1.	Scorbutic	9340.	5420.	25,810	26,900	23,430.	20,625	19,840.	16,250.	11,100	12,240.	survived
21.	Non scorbutic	8670.	5204.	18,730	16,330.	13,360.	10,700	6,440.	7,280.	5,420.	4110.	died
13.	Scorbutic	9840.	6470.	5,810.	3,430.	died	-----	-----	-----	-----	-----	-----
5.	Non Scorbutic	10,930	6220.	11,800	22,500.	21,220	25,450	15,460.	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 later stage of the disease and usually portends a fatal result. The primary/

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 interference with the occurrence of a leucocytosis in the peripheral blood stream. These experiments show that when small doses of pneumococci - 500 million - guinea-pigs, both healthy and scorbutic animals 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 & 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/

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 septicaemia, 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.

TABLE VII

Differential leucocyte counts in guinea-pigs inoculated intraperitoneally with 100 million pneumococci.

Date.	Polymorphonuclears.	Eosinophils	Basophils.	Lymphocytes	Mononuclears	Myelocytes	Dead.
Guinea-pig 16.- Scorbutic.							
Before injection	42.	4	1	46.	7	-	-
3 hours after inject	48.	1	2	45.	4	-	-
1 day " "	55	1	3	32.	8	-	-
2 days " "	56.	4	2	27.	9	-	2
3 days " " (animal moribund)	32	1	3	55.	6.	2	4
Guinea-pig 20 - Non-scorbutic							
Before injection.	47	2	0	43.	8	-	-
3 hours after injection	41	2	0	54	5	-	-
1 day " "	57.	1	2	34.	6	-	-
2 days " "	58.	2	3	30	7	-	-
3 days " "	51.	1	1	43.	4	-	-
4 days " "	49.	1	2	40	8	-	-
5 days " "	54.	1	3	35.	5	-	2
6 days " "	40.	2	2	48.	6	-	2
7 days " "	36.	1	2	47	7	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 (1911)[35] as the result of a recent study of the effects of hemolytic streptococci on the blood and haemopoietic organs of rabbits has come to the conclusion that the occurence of a leucopenia coupled with the presence of myelocytes in the peripheral blood stream is of very grave importance. In the scorbutic animals which suffered from pneumococcal septicaemia 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. Brugsh and Schilling (1908)[36] consider the presence of these polymorpho-nuclear cells with rod-shaped nuclei to be very characteristic of acute pneumonic infections. In order still further to compare the effects of the

pneumococcal/

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 day 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 septicaemia there was a very moderate reaction on the part of the leucoblastic cells, as shown in Fig.7&8. 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. 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 septicaemia the degenerative changes were well seen. In the myelocytes the/

the protoplasm was more granular than usual, while karryoxhexis, 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 septicaemia 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 to choose 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/

require to be carried out on a far more extensive scale before absolutely accurate 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. 100 million, the resistance of the scorbutic animal breaks down more rapidly than that of the control. The scorbutic animal dies from an acute pneumococcal septicaemia, while the healthy animal dies after a somewhat longer period of time with a localised pneumococcal septicaemia. 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 toxin may be at work. Though attempts to isolate intracellular toxins from the pneumococcus have not been very satisfactory, Cole (1921) has recently claimed to have obtained a toxin with specific effects by lysing the pneumococci with bile. The results which he obtains by injecting the toxin intravenously into guinea-pigs are, however, too uncertain for any accurate quantitative work to be carried out. The rapidity with which fatty degeneration appears in the heart muscle and degenerative changes/

changes occur in the bone marrow in scorbutic animals suggests either that these organs are 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[38]) 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. In the dog Tchistovitsch and Jurevitsch (1908[39]) have also described phagocytosis of pneumococci. Wadsworth (1912[40]) 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 Klein (1915[41]) have shown that in rabbits rendered aplastic with benzol the resistance to pneumococcal infection is greatly reduced. In

chronic/

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 septicaemia 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.

III. 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 111.

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 the following table.

TABLE VIII.

The effects of intra-peritoneal injections of Staphylococcus aureus in guinea-pigs.

Number of Animal.	Condition of Animal.	Weight in grams.	Number 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.	–	" " "
42.	"	275.	10,000.	–	
43.	"	244.	20,000.	5	peritonitis. Staphylococci present
44	"	252.	20,000.	6.	" " "
45	Non-scorbutic	247.	5,000	–	
46.	"	254.	5,000.	–	Killed after 10 days: no peritonitis
47.	"	225.	10,000	–	
48.	"	285.	10,000.	–	" " "
49.	"	296.	20,000.	–	
50.	"	302.	20,000.	–	" " "
51.	"	295	50,000.	3.	peritonitis. Staphylococci present
52	"	260.	50,000.	3.	" " "

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/

peritonitis, though no organisms 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 5th and 6th 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/

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.

TABLE IX.

Total and differential leucocyte counts in guinea-pigs inoculated intraperitoneally with 20,000 million staphylococci.

Guinea-pig No. 43. Scorbutic.

Date.	Number of leucocytes per c.mm.	Polymorpho nuclears	Eosinophil	Basophils	Mono-nuclears	Lympho-cytes.	Myelo-cytes.	Dead.
Before injection.	8180.	42.	4	1	7	46.	-	-
3 hours after inject.	4060.	46.	0	2	4	48.	-	-
1 day " "	10,150.	55.	1	3	8	33.	-	-
2 days " "	14,680	57.	4	2	9	27.	-	1
3 days " "	13,430.	50.	5	1	9	34.	-	1
4 days " "	12,500.	60	1	0	6.	32.	1	1
5 days " "	3,200.	40	1	2	8	46.	1	2

Guinea-pig No. 45 - Non-scorbutic.

Before injection.	9560.	43	1	1	2	53.	-	-
3 hours after injection	3750.	43	0	2	6	49	-	-
1 day " "	17,180.	69.	0	0	3	28	-	-
2 days " "	10,250.	44.	0	3	8	45.	-	-
3 days " "	12,120.	34.	1	1	6	58	-	-
4 days " "	13,400.	26	1	2	9	60.	-	-
5 days " "	10,250	36.	2	1	6.	55.	-	-

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 red 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)[42] 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.

These experiments though less extensive and less suggestive than those with the pneumococcus nevertheless tend to confirm the suggestion that the scorbutic guinea/

guinea-pig is more susceptible to the action of bacterial infection than the healthy animal.

III. Streptococcus haemolyticus.

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

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 as shown in the following table:-

TABLE X

The effects of intraperitoneal injections of Streptococcus haemolyticus in guinea-pigs.

Number of Animal.	Condition of Animal.	Weight in grams.	Number of streptococci in millions	Period of survival in days	Remarks.
55.	Scorbutic	265.	100	-	Killed after 10 days. no peritonitis.
56.	"	280.	100	-	
57.	"	245.	200	-	" " "
58	"	302.	200	-	
59	"	255.	500	3	a small amount of peritoneal exudate: streptococci from heart blood.
60	"	260.	500	2	
61.	"	284.	1000	4	
62	"	240.	1000	2	
63	"	245	2000	3	
64	"	259.	2000	3	
65.	Non-scorbutic	240.	100	-	killed after 10 days: no peritonitis
66.	"	276.	100	-	
67.	"	308	200	-	" " "
68	"	315.	200.	-	
69.	"	254.	500	-	" " "
70.	"	260.	500.	-	
71.	"	252.	1000.	3	a small amount of peritoneal exudate: streptococci from heart blood.
72	"	235.	1000.	4	
73.	"	246	2000.	2	
74.	"	259.	2000.	2	

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 & 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 post mortem examination of the scorbutic guinea-pigs dying from streptococcal peritonitis was the number of petecchial haemorrhages, which were found on the surface of the peritoneum, pleura and round the knee joints. These haemorrhagic foci were all quite recent. As was previously mentioned in discussing the pathological changes in chronic scurvy, it is not common to find numerous haemorrhages in the chronic form of the disease in the guinea-pig. These haemorrhagic 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 haemorrhages, only one of the controls - No. 72 - showed a few haemorrhagic/

haemorrhagic 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.

(IV) 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 the following table:-

TABLE XI.

The effects of intraperitoneal injections of bacillus coli in guinea-pigs.

Number of Animal.	Condition of Animal.	Weight in grams.	Number of b. coli in millions	Period of Survival in days.	Remarks
77.	Scorbutic	310	200.	–	Killed after 10 days: no peritonitis
78.	"	345	200.	–	
79.	"	360.	700.	3	Peritonitis: b.coli from heart blood
80.	"	352	700.	3	" " "
81.	"	330.	1000	2	" " "
82.	"	326.	1000.	1·5.	" " "
83.	Non-scorbutic	315	200	–	Killed after 10 days: no peritonitis
84.	"	320.	200.	–	
85.	"	345.	700	–	" " "
86.	"	312	700	–	
87.	"	332	1000	2	Peritonitis: b.coli from heart blood
88.	"	365.	1000.	2.	" " "

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 b.coli infections showed numerous haemorrhagic areas, while the control animals showed none.

The changes in the leucocytes of the peripheral blood stream/

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.

The result of these experiments with four species of bacteria shows definitely 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 toxaemia 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 more toxin is formed by the bacteria. The definite rupture in the defence mechanism of the body appears to be due to degenerative changes in the bone marrow resulting primarily from the congestion and haemorrhage present in the marrow, as the result of a diet deficient in vitamin C. Clinically the symptoms of chronic guinea-pig scurvy are almost unnoticeable. It is only when a superadded bacterial infection is present that the weakness in the defence mechanism becomes apparent. Chronic scurvy therefore provides another instance of the well-known fact that degeneration in the haemopoietic bone marrow is associated with a reduced resistance to bacterial infection.

11. DEFICIENCY IN VITAMIN B and BACTERIAL INFECTION.

(a) The Blood and Haemopoietic Organs in Beriberi.

The general histological changes in the tissues which result from a deficiency of vitamin B in the food have now been so extensively investigated both in man and in animals that any further description would be superfluous. Nevertheless it is surprising that up to the present very little attention has been directed to the haemopoietic organs and the cellular changes in the blood in beriberi.

(1) The blood cells in man do not show any very great deviation from the normal. Fayrer (1880)[43] described a great diminution in the number of red cells, the blood picture being not unlike that found in pernicious anaemia. At that time, however, ankylostomiasis was frequently confused with beriberi. More recent work by Vedder (1913)[44] has shown that there is only a moderate degree of anaemia. In sixteen cases in Europeans examined by the writer the average number of red cells was 4,200,000 per cmm, while Marshall (1911)[45] found no anaemia in six Chinese coolies with beriberi. Polychromatophilia and pyknosis are not commonly found.

Very few observations have been made on the leucocytes in human beriberi. Findlay (1920)[46] found the average count/

count to be 5,500 per c.mm. The results of differential counts obtained by different observers vary considerably. Thus Bezancon and Lakke (1906)[47] could find no marked deviation from the normal. Takasu (1904)[48] on the other hand found the number of neutrophils always lower than the number of lymphocytes. Similar results were obtained by Saltet and Legrand (1909)[49].

Mathis and Leger (1911)[50] as the result of a prolonged study of forty cases of beriberi in Madagascar came to the following conclusions:-

(1) The number of neutrophil polymorphs was normal six times (64 to 69 per cent), diminished 34 times (64 to 44.3 per cent) the average was 57 per cent.

(11) The average lymphocyte count was 36.

A possible explanation of these discrepant findings has been pointed out by Chamberlain and Vedder (1911)[51] who have shown that in the tropics native races have a relatively higher number of lymphocytes than the inhabitants of cooler climates. Europeans, however, after a few years residence in the tropics also tend to show a relative lymphocytosis. It is possible therefore that the lymphocytosis observed in beriberi by such observers as Mathis and Leger is more apparent than real.

Findlay (1920)[46] found that in Europeans the differential count scarcely deviated from the normal. The average

for/

for sixteen cases was:-

Neutrophils	-	64 per cent.
Lymphocytes	-	25 per cent.
Mononucleears	-	8 per cent.
Eosinophils	-	3 per cent.

The proportion of adult and dead to young neutrophils was increased. The exact significance of this shift to the right is at present unknown.

In animals suffering from beriberi there have been comparatively few observations on the leucocytes of the blood. A few investigations have, however, been carried out both on pigeons and rats fed on diets deficient in vitamin B.

When pigeons are fed on a diet of polished rice they develop well marked nervous symptoms within three or four weeks. A diet of polished rice is of course deficient not only in vitamin B but in vitamins A and C. The addition of small doses of cod liver oil and orange juice to the polished rice dietary is however without any effect on the occurrence of the beriberic symptoms.

McCarrison (1921)[52] has found that in pigeons with beriberi there is a reduction in the number of the red cells. Thus in control birds the range of variation was from 2,720,000 to 4,752,000 per c.mm., while in pigeons with beriberi the variation was from 1,232,000 to 3,452,000 per c.mm. The average for control birds was 3,599,733 per c.mm as compared with 2,763,500 per c.mm in diseased birds/

birds. No marked diminution or alteration in the proportion of the white cells could be definitely established in the pigeons suffering from beriberi. These observations have been confirmed by observations on ten control and ten beriberic pigeons. In the controls the range of variation was from 2,440,000 to 4,950,000 per c.mm, while in beriberic pigeons it ran from 1,540,000 to 3,870,000 per c.mm. The average for controls was 3,724,000 per c.mm as compared with 3,050,000 per c.mm in diseased birds. Such marked fluctuations were present from day to day in the leucocyte counts even of normal birds that no data of any value could be obtained either from total or differential leucocyte counts in the pigeon.

In rats fed on a diet deficient in vitamin B very definite changes in the leucocytes have recently been recorded by Cramer, Mottram and Drew (1921)[53]. According to these observers in the final stages of the disease there is a fall in the number of lymphocytes from an average figure of 8000 per c.mm in the normal rat to a figure as low as 1200 per c.mm in the beriberic animal. The polymorphonuclear leucocytes are not affected to the same extent. The blood counts in normal rats, as found by various workers, are shown in the following table:-

TABLE XII

Showing the blood counts in normal rats.

Observer.	No. of leucocytes per c.mm.	Polymorpho nuclear leucocytes.	Eosino phils.	Baso phils.	Lympho cytes	Mono nucleers	No. of red cells per c.mm.
Goodall (1910[14]).	10,600.	28	3	1	68	–	8,100,000.
Yakimoff (1911[58])	8-13,000.	20-36.	0·6-5·6	–	58-74.	–	7-8,600,000
Author	7-12,800.	22-34 29.	0·5-6·0 2	–	54-70 55.	8-14 10.	6,400,000-8,720,000. 7,640,000.

Observations were carried out on the blood of ten rats when actually suffering from the paralytic symptoms produced by a diet lacking in vitamin B. The diet on which the animals had been fed consisted of

Caseinogen	20 grs.
Starch	55 grs.
Cod liver oil	15 grs.
Salt mixture	5 grs.
Orange juice	5 c.cs.

The salt mixture was that recommended for the nutrition of rats by McCollum and Davis (1915[55]). Ten control rats were given the same diet as the above but with the addition of 5 grs. a day of autolysed yeast/

yeast,- marmite. The results obtained are shown in the following table:-

TABLE XIII

Showing the blood counts in rats fed on a diet deficient in vitamin B and in controls.

Number of Animal.	Condition of Animal	Number of Red cells per c.mm.	Number of leucocytes per c.mm.	Polymorphonuclear leucocytes	Eosinophils	Basophils	Mononuclear	Lymphocytes
1	-vitamin B	7,400,000.	6,240.	55.	2	0	8	35
2	"	6,220,000.	4,320.	62.	1	1	7	29
3	"	6,400,000.	3,400.	60	3	0	4	33.
4	"	5,800,000.	7,600.	67	0	0	9	22
5	"	7,060,000.	4,100.	48	2	0	6	44
6	"	8,090,000.	2,900.	53	1	0	5	41
7	"	5,600,000	5,370.	57.	5	1	4	33
8	"	6,650,000.	6,900.	66	4	0	6	24
9	"	6,080,000.	3,650.	69	0	0	6	25
10	"	6,100,000.	6,400.	45	3	0	5	47.
11.	+ vitamin B	6,640,000.	8,300.	40	3	0	6	51
12	"	8,500,000.	8,640.	35	1	0	4	60
13.	"	7,470,000.	10,240.	25	6	0	5	70.
14	"	7,270,000.	9,370.	38.	3	0	5	54
15	"	8,650,000.	11,540.	36	2	1	6	55.
16	"	8,400,000.	7,600.	29.	0	0	8	63
17	"	8,260,000.	12,800.	37	2	1	6	54.
18	"	7,940,000.	10,490.	42.	0	0	5	53
19	"	7,930,000	9,850.	36	0	0	3	61.
20	"	7,520,000	10,300.	28.	1.	1.	9	61.

These results largely confirm the findings of Cramer and his co-workers. There is a very slight reduction in the red cell count, the average being 6,540,000 per c.mm. There is quite a definite reduction in the number of leucocytes, both of lymphocytes and to a less extent of polymorphonuclear cells, as a result of which the differential leucocyte count shows a great preponderance of polymorphonuclear cells.

The examination of stained blood films showed a very large number of polymorphonuclear leucocytes with fragmented nuclei and with vacuolated protoplasm. This shifting of the index to the right in the leucocyte picture of rats suffering from a deficiency of vitamin B has also been noted by Happ (1921)[56]. It is similar to what occurs in human beriberi.

(11) The Bone Marrow. Practically no observations appeared to have been made on the condition of the bone marrow in human beriberi.

In pigeons McCarrison (1921)[57] merely mentions that the bone marrow is decreased in amount in beriberic birds. He does not, however, enter into any details as to the exact pathological changes. An examination of the bone marrow in pigeons dying from beriberi nevertheless shows very definite changes. The bone marrow is lighter in colour than the normal and on microscopical examination there are seen to be considerable areas

in which gelatinous degeneration has occurred with the disappearance both of leucoblastic and enythroblastic cells. The appearance of the bone amrrow is very similar to that described by Jackson (1904[57]) as seen in the bone marrow of starved birds, though in the beriberic birds there is rather more congestion than in those subjected to complete inanition.

In rats fed on a diet of casein, starch, cod liver oil and inorganic salts, but lacking orange juice and yeast there are seen two noticeable changes in the bone marrow. The capillaries are intensely congested and at certain points appear to have ruptured. At other points there is an appearance of early gelatinous degeneration with a reduction in the number of the haemopoietic cells. As McCollum (1922[58]) and his co-workers have recently pointed out these changes in the bone marrow of rats fed on diets deficient in vitamin B are very similar to those occurring in the bone marrow of guinea-pigs suffering from acute scurvy. The changes seen in the bone marrow are illustrated in Fig. 11 . The addition of orange juice to the diet does not prevent these degenerative changes in the marrow so that they cannot be looked upon as being actually caused by a deficiency of vitamin C.

(111) Lymphatic tissues. Thymus. In children suffering from the infantile form of beriberi there does/

does not appear to be any marked atrophy of the thymus. Thus Miura (1899[59]) stated that the thymus is of normal size, while McLaughlin and Andrews(1910[60]) and Andrews (1912[61]) in their exhaustive papers find that the thymus is usually prominent and full while microscopically there is possibly an increase in the cellular elements. Hassall's corpuscles appear normal. In infants beriberi is an acute disease and inanition is not marked since most of the infants seemed to be well nourished with a fair amount of subcutaneous fat. In pigeons there is almost complete disappearance of the thymus as the result of a diet of polished rice, a fact first noted by Funk and Douglas (1914[62]) and later confirmed by McCarrison (1921[52]). Atrophy of the thymus also occurs in hens fed on the same diet and as Findlay (1921[63]) has shown is met with in birds subjected to complete inanition.

In rats Cramer and his co-workers (1921[53]) have described a very similar atrophy as occurring from a diet deficient in vitamin B.

Spleen and Lymph glands. In man in very chronic cases there is not uncommonly some atrophy of the spleen and lymph glands though in the more acute cases atrophy is not a prominent feature. Scheube (1884[64]) found that the spleen was usually enlarged, while Miura (1888[65]) noted that the spleen was usually of normal size, colour and consistency, though sometimes atrophic and rarely/

rarely enlarged. Yamagiwa (1899) found a distinct perceptible increase in size in the majority of cases. Splenic atrophy or atrophy of the lymphoid tissue generally has not been noted in infantile beriberi. In pigeons and hens the atrophy of the spleen and lymphoid tissue generally is a striking feature which is also well seen in rats.

Atrophy of the lymphoid tissues of the body is thus a very characteristic occurrence in experimental animals fed on diets deficient in vitamin B. It must however be remembrerd that in these animals there occur very marked symptoms of general inanition characterised by a great loss of body weight. In man, except in very chronic cases, this loss of body weight is not by any means constant, and in association with this, atrophy of the lymphoid tissue is also not a constant pathological finding.

Evidence will be brought forward to show that atrophy of lymphoid tissue also occurs in association with diets, which though containing an ample supply of vitamin B, are nevertheless deficient in some other essential respect.

(b) Bacterial Infection and Beriberi.

For many years it was believed by many authorities that beriberi was an infectious disease due to some micro-organism. This view was based on the frequency with which organisms were isolated either from the blood/

blood during life or from the tissues after death in cases of beriberi. Through the organismal theory of beriberi is now of historic interest only, it is nevertheless of importance as showing the ease with which the tissues of beriberic animals are attacked by bacteria. All those who have fed animals on diets deficient in vitamin B have been struck by the number of animals which despite the best hygenic conditions, nevertheless die from some secondary bacterial infection, while McCarrison (1921)[52] has also noted that monkeys fed on a diet deficient in vitamin B are very liable to attacks of amoebic dysentery, although control monkeys, parasitised with Entamœba histolytica remained in good health.

In order to determine the extent of the rupture in the defence mechanism resulting from a deficiency of vitamin B experiments were carried out on pigeons with the pneumococcus. As is well known the pigeon is practically immune to the action of the pneumococcus even when introduced intraperitoneally in relatively enormous doses. The pneumococci have completely disappeared from the peritoneal cavity in from three to eight hours. The cause of this immunity has not been satisfactorily explained, though there is some evidence to show that the pneumococcus rapidly dies off at 107 -108° F temperatures, which are normal for the healthy pigeon. Strouse (1909)[67] acting on this suggestion/

suggestion reduced the pigeon's temperature by subcutaneous injections of pyramidon and then injected pneumococci intraperitoneally with the result that the bird died in a few hours with a marked pneumococcal peritonitis.

These experiments with pyramidon were repeated. A normal pigeon, with a cloacal temperature of 106.2° F was given a subcutaneous injection of 0.45 grams of pyramidon. Two hours and a half later when its temperature was 101.2° F it was inoculated with four blood agar slope cultures of pneumococcus suspended in saline. The pneumococcus, which was the same strain as that used for the injections of guinea-pigs was grown for twenty-four hours only before injection. The pigeons died thirty-one hours later and on post-mortem examination showed an extensive exudate into the peritoneal cavity. The exudate which was swarming with pneumococci contained a large number of polymorphonuclear leucocytes. There was, however, no phagocytosis of pneumococci going on. The pneumococcus was also recovered in pure culture from the heart blood. The administration of 0.45g of Pyramidon subcutaneously or the intraperitoneal injection of the same dose of pneumococci is without any effect on the pigeon, as is seen from a reference to Chart 1M.

It is well known that one of the effects of a diet of polished rice on birds is to produce a lowering of the body temperature, whether by a direct action on the central/

central nervous system or by its slowing effect on all metabolic processes is at present unknown. This reduction in the body temperature begins during the first week of the polished rice diet. Four pigeons were placed on the polished rice until their temperatures were reduced to between 103° - 104°F. They were then each inoculated intraperitoneally together with four control birds, with six blood agar slopes of the pneumococcus. Death took place in the beriberic birds in from nine to thirty-six hours after the inoculation while the control birds showed no untoward symptoms. None of the birds on the polished rice diet showed any signs of paralysis either before or after the inoculation of pneumococci. The temperatures of the beriberic and control pigeons are seen in Charts ZV - Vlll. In each instance the beriberic birds showed a considerable amount of exudate into the abdominal cavity, while the pneumococcus was isolated in pure culture from the heart blood.

These experiments definitely show that diet can render an animal susceptible to an organism to which under normal circumstances it is entirely immune. The reduction in temperature would seem to be the main factor in decreasing the resistance, though possibly the degenerative changes in the bone marrow of pigeons fed on polished rice may also play some part, since it was noticeable that in the beriberic birds the leucoblastic reaction in the bone marrow was less active/

active than in the pigeon with its temperature reduced by pyramidon. Further experiments are at present being conducted on the resistance of pigeons on a deficient diet to other species of bacteria. At present of course it is not possible to apply these results to man though it is of interest to note that in human beriberi the temperature is almost always subnormal.

DEFICIENCY IN VITAMIN A
AND
BACTERIAL INFECTION.

During the past few years the effects of diets lacking in vitamin A have received very considerable attention. Apart from its importance as a growth factor, the lack of vitamin A would appear to be in some way connected with an eye condition - Keratomalacia - which appears promptly when the diet is deficient in vitamin A and disappears after the administration of substances containing vitamin A. Keratatomlacia has been chiefly studied in rats fed on diets deficient in Vitamin A. The curious thing is, however, that keratomalacia only occurs in a small per centage of animals fed on a diet lacking in this factor. Thus, out of twenty-four young rats fed on a diet complete in every way, except that it lacked vitamin A, only six developed keratomlacia, which appeared after from fifty to seventy-five days' feeding. In two of the animals the lesion was monocular Attempts to affect the sound eye with material taken directly/

directly from the infected one were unsuccessful, thus confirming the recent experiments of Walker (1922)[68]. Attempts were also made to infect the eyes of other rats which, though on a diet deficient in vitamin A, were not suffering from keratomalacia. These experiments were also unsuccessful. From the conjunctival exudate there were isolated streptococci, staphylococci and a small Gram negative bacillus. An exactly similar flora was isolated from the conjunctivae of rats on the diet lacking in vitamin A, but showing no eye symptoms, and from the conjunctivae of control rats. The onset of the condition is usually quite sudden, and prior to the appearance of the keratomalacia it is not possible to determine by by ~~the~~ histological methods any changes in the cells of the corneal conjunctiva. The earliest histological changes consist in a proliferation of the cells of the corneal conjunctiva, later there is hyalinisation or necrosis of the outer layers of corneal epithelium, accompanied by an exudation of serum and an infiltration of small round cells. According to Wason (1921)[69], the anterior chamber of the eye may be involved in advanced cases. Keratomalacia has been recorded by Block (1917)[70] as occurring in epidemic form among children in Denmark, while Wells (1920)[71] has noted its occurrence among the famine sufferers of/

of Roumania. In human beings, as in rats, the condition promptly clears up on the addition of a sufficient ration of vitamin A to the food. It is of interest to note that Mellanby (1921[72]) has recently noticed the occurrence of keratomalacia among puppies fed on a diet deficient in the "anti-rachitic" vitamin which may or may not be identical with vitamin A.

The majority of observers believe that keratomalacia only occurs in animals or man when the diet is deficient in vitamin A, though recently Funk and Dubin (1920[73]) have recorded its occurrence in two out of thirty rats fed on a diet deficient in vitamin B. As Bulley (1919[74]) has pointed out, however, it is of importance to distinguish clearly between keratomalacia and simple eye infections, such as may occur in any poorly nourished laboratory animal. The same observer has also shown that keratomalacia in rats may be entirely prevented by washing out the conjunctival sac with a solution of some dilute disinfectant.

The occurrence of keratomalacia would thus seem to be dependent on at least two factors,-(1) a deficiency of vitamin A in the food, and (2) the presence of a suitable bacterial flora. The question, however, arises as to whether these two factors are really sufficient to explain its highly irregular occurrence, since keratomalacia/

keratomalacia is not usually associated with any generalised septicaemic process. In the rats, which in my experiments suffered from keratomalacia, it was noted that in four cases, death took place within a week of the onset of the eye symptoms and in each case the lungs showed marked evidence of broncho-pneumonia.

(A) THE BLOOD AND HAEMOPOIETIC ORGANS IN VITAMIN A DEFICIENCY.

Although the exact part which vitamin A plays in the etiology of rickets cannot be at present definitely stated, there is considerable evidence to show that cod-liver oil, which is extremely rich in vitamin A, plays some rôle in the deposition of calcium salts in cartilage. In view of the possibility of the relationship of vitamin A to rickets, it is therefore of some interest to compare the known facts regarding the blood and haemopoietic organs in rickets with those observable in the condition resulting from a deficiency of vitamin A.

No observations appear to have been made on the blood of human beings suffering from keratomalacia. In rats, however, Cramer, Mottram and Drew (1922) have investigated the changes in the blood, spleen, and thymus/

thymus. These authors found only a slight reduction in the number of lymphocytes in the peripheral blood stream, accompanied by a slight reduction in the size of the spleen and thymus. In order to investigate this question somewhat more fully, twelve young male rats of roughly the same weight were placed on the following diet:-

Caseinogen - - - - - - -	20grs.
Starch - - - - - - - -	55grs.
Cotton-seed oil - - -	15grs.
Autolysed yeast - - -	5grs.
Inorganic salt mixture	5grs.

while six control rats of the same age, sex and weight were placed on a similar diet, except that cod-liver oil replaced the cotton-seed oil.

The rats fed on the diet lacking in vitamin A died in from 57 to 99 days. Two of them showed signs of keratomalacia, while at the post mortem examination these two and one other were found to be suffering from broncho-pneumonia. These three rats were therefore not included in the tables illustrating the changes in the blood and lymphatic tissues since it was considered probable that the blood picture might be vitiated by the infective process. The heart blood of the other nine rats was sterile. Particulars of/

of the red and white cell counts are given in Table. The blood counts here recorded were taken after the animals had been on the diet for a period of eight weeks.

In order to avoid any personal bias in making the total and differential counts, the blood was obtained by an assistant and given to me without any knowledge on my part as to whether it came from an animal on the control or on the deficient diet.

TABLE XIV.

showing the blood changes in rats fed on a diet deficient in vitamin A.

No. of animal	Condition	No. of red cells per c. mm.	No. of white cells per c. mm.	Polymorphonuclear leucocytes.	Eosinophils.	Basophils.	Lymphocytes.	Mononuclears.
33.	- vitamin A.	8,200,000.	9,200	54.	1.	0.	42.	3.
34.	"	7,400,000.	7,420.	62.	0.	0	36.	2.
35.	"	8,500,000.	8,640.	49.	0.	0.	47.	4.
36.	"	6,820,000.	5,730.	56.	1.	1.	37.	5.
37.	"	7,220,000	6,840.	51.	2.	0	43.	4.
42.	"	9,140,000.	10,170.	46.	1.	0.	50.	3.
43.	"	7,320,000	8,600.	55.	0.	0.	39.	6.
44.	"	8,500,000	9,470.	50.	0.	0.	42.	8.
49	"	7,250,000	7,500.	47.	1.	0.	47.	5.
73.	+ vitamin A	7,430,000	8,860.	40.	3.	0	54.	3.
74.	"	8,600,000	9,730.	25.	0.	0.	69.	6.
75.	"	8,470,000	11,150.	35.	1.	0.	60.	4.
76.	"	7,930,000	7,300	38.	3.	0.	54.	5.
77.	"	8,290,000	10,250.	29.	0.	0.	64.	7.
78.	"	7,350,000	9,450.	32.	1.	0.	62.	5.

It will be seen that there is practically no reduction in the number of red cells in the peripheral blood stream, a point of some interest since Leonard Findlay (1909)[75] has shown that the supposed anaemia of rickets is non-existent. The number of leucocytes is in many cases normal, but in a few instances there is a definite reduction, while the differential leucocyte count shows a decreased number of lymphocytes in the peripheral blood stream. This decrease is not quite as marked as in the case of rats deprived of vitamin B.

The changes in weight in the thymus and spleen are shown in the Table. XV Both the absolute weight of the organ is recorded and the weight of the organ calculated per 100 grammes of original body weight. The control animals, which, of course, had increased in weight on the normal diet, were all killed on the seventieth day of the experiment.

TABLE XV.

showing changes in weight in spleen and thymus in control rats and in rats on a diet lacking vitamin A.

No. of animal.	Weight in grs. at beginning of experiment.	Weight in grs. at end of experiment.	Duration of life in days.	Actual Wt. of Spleen in mgs.	Wt. of Spleen in mgs. per 100grs of original body weight.	Actual Wt. of Thymus in mgs.	Wt. of Thymus in mgs. per 100 grs of original body weight.
33-vit. A.	138.	116.	57.	220.	159.	140.	101.
34. "	120.	96.	67.	210.	175.	150.	125.
35. "	150.	110.	73.	270.	180.	120.	80.
36. "	148.	130.	76.	320.	216.	190.	128.
37. "	144.	112.	77.	200.	138.	140.	97.
42 "	128.	92.	99.	220.	171.	110.	85.
43. "	110.	82.	79.	150.	136.	90.	82.
44. "	146.	92.	92.	200.	137.	100.	68.
49 "	178.	92.	96.	210.	117.	110.	81.
73 + vit. A	110.	182.	70.	820.	745.	360.	327.
74.	105.	170.	70.	840.	800.	450.	428.
75.	93.	135.	70.	920.	989.	510.	548.
76.	87.	125.	70.	760.	873.	460.	529.
77.	99.	145.	70.	980.	989.	550.	555.
78.	110.	178.	70.	780.	709.	480.	436.

It will be seen that there is quite a definite reduction in the size of the spleen and thymus as the result of a diet deficient in vitamin A, though it is not quite so well marked as on the diet deficient in vitamin B. The reduction in the size of the spleen and thymus and the atrophy of the lymphoid tissues generally as the result of a diet lacking vitamin A has also been noted by McCellum, Park, Shipley, Powers and Simmonds (1921)[76]. They have furnished a possible explanation of the discrepancies in the findings of different workers in regard to the extent of the lymphoid atrophy, since they have found that when rats fed on a diet deficient in vitamin A are exposed to sunlight, though keratomalacia may still occur, nevertheless their general condition remains much better and the lymphoid atrophy is much less marked than in similarly fed rats kept in a dark place.

Microscopic examination of the spleen and thymus showed a reduction in the number of lymphoid cells, while mitotic figures were very rare. In the present state of our knowledge it is very doubtful whether any very acurate conclusions can be drawn from the atrophy of the thymus since so little is known in regard to its involution in thehealthy rat. The decrease in the size of the spleen is, however, of importance as indicating/

indicating the general atrophy of the lymphoid tissue. It does not, however, appear to be possible to look upon the atrophy of the lymphoid tissue as a specific result of a deficiency of vitamin B but rather as a change which occurs in any process of atrophy, including that of old age.

Examination of the bone marrow in animals fed on a diet lacking in vitamin A has not shown the occurrence of any very constant change. In quite a number of cases, however, there are patchy areas of gelatinous degeneration and in two instances the gelatinous degeneration was very widespread.

Animals living on a diet deficient in vitamin A rarely die from a general septicaemic process, but very frequently from broncho-pneumonia. This broncho-pneumonia is frequently associated with the presence of bacillus bronchisepticus in the lungs, since this organism was present in every case examined, while in two out of eight cases there was present in one a pneumococcus, in the other a non-haemolytic streptococcus. These three organism have, however, been isolated from the throats of apparently healthy rats living in the animals houses of this laboratory. The question therefore arises as to whether the broncho-pneumonia occurring in animals fed on a deficiency of vitamin A is not comparable to the keratomalacia/

keratomalacia which appears in these animals, since, as in the case of keratomalacia, it is noticeable that only a certain percentage of rats fed on this deficient diet develop broncho-pneumonia. In addition to the deficiency of vitamin A and the presence of an appropriate bacterial flora there may therefore be a third factor necessary to induce the onset of the pneumonic process.

(B) THE OPSONIC POWERS OF THE SERUM OF ANIMALS ON A DIET DEFICIENT IN VITAMIN A.

In order to supplement the experiments carried out on the opsonic powers of the serum in animals suffering from a deficiency of vitamin C, a few observations were carried out on the opsonic qualities of the serum in rats fed on a deficiency of vitamin A. The sera of four rats which had been fed for ten weeks on a diet lacking in vitamin A was collected and compared with that of four control rats. The technique used was the same as with the scorbutic animals, the leucocytes employed being those of a healthy control rat. The two lots of sera were pooled before use and the opsonic activity determined for two types of organism,-bacillus coli and staphylococcus aureus. The data are shown in Table.

TABLE/

TABLE XVI.

showing the opsonic activity of the serum of rats on a diet lacking vitamin A and of control rats.

Serum.	Organism.	No. of polymorpho. nuclear leucocytes examined.	No. of polymorpho. nuclear leucocytes containing bacteria.	Total no. of bacteria phagocytosed.	Phagocytic index.
-vitamin A	Staphylococcus	100.	79.	128.	1·28.
	B. coli.	100.	48.	46.	0·46.
+ vitamin A	Staphylococcus.	100.	84.	136.	1·36.
	B. coli.	100.	52.	49.	0·49.

It will be seen that there is no significant difference in the readings obtained with the two sera and thus little evidence for a decrease in the opsonic activity of the serum as the result of a diet deficient in vitamin A.

DIETS DEFICIENT IN PROTEIN

The changes in the blood and haemopoietic organs.

Although during the past few years the liability to infection of animals fed on vitamin free diets has been especially emphasised by numerous workers, there are/

are not wanting observations to show that other dietary deficiencies may also lower the resistance to bacterial infection. Thus, McKay (1912) in India has found that certain native races which live on a low protein diet have a poor physique and succumb to infection more readily than other races which live on a high protein diet. In the following experiments on rats the effects were investigated of a diet deficient in amount in protein, special attention being paid to the changes in the blood and haemopoietic organs. Twelve growing rats were placed on the following food mixture in which only a third of the usual amount of caseingen was included, though in other respects it was complete:-

Caseingen	7grs
Starch	55grs.
Cod liver oil	15grs.
Marmite	5grs.
Salt mixture	5grs.
Orange juice	5ccc.

As a result of this diet the weights of the rats at first remained stationary ~~and then~~ began to decrease slowly ~~in weight~~. After four months on the diet the blood changes were investigated, ~~after which~~ subsequently the animals were killed and the weights of the thymus and spleen recorded/

recorded. The same precautions in regard to the blood counts were taken as in the case of the animals on a diet deficient in vitamin A.

The changes in the blood cells and in the weights of the spleen and thymus are shown in Tables XVII and XVIII.

TABLE XVII

showing changes in the blood cells of rats fed on a diet deficient in amount in protein.

No. of animal.	No. of red cells per c. mm.	No. of white cells per c. mm.	Polymorpho-nuclear leucocytes.	Eosinophils	Basophils	Lympho-cytes.	Mono-nuclears.
61.	7,250,000.	6,500.	49.	1	0	44.	6.
62.	8,500,000.	7,300.	53.	1	0	41.	5.
63.	6,940,000.	6,950.	52.	1	0	42.	5.
64.	7,440,000.	8,520.	44.	1	0	51.	3.
65.	8,200,000.	5,800.	53.	0	0	43.	4.
66.	7,560,000.	6,240.	50.	1	0	46.	3.
67.	6,840,000.	7,120.	53.	1	0	41.	5.
68.	8,250,000.	8,660.	43.	2	0	49.	6.
69.	7,450,000.	7,430	51.	0	0	45.	4.
70.	8,500,000.	5940.	53.	1	0	41	5.
71.	6,900,000	6260	47.	1	0	48	4.
72.	7,580,000	7280	53.	1	0	42.	4.

In some cases there will be seen a reduction in the number of the red cells, but in the majority of cases there is practically no anaemia. There is, however, a slight and almost constant reduction in the number of leucocytes, when compared with the controls shown in Table XV , while the differential leucocyte count shows very similar changes to those observed in the rats fed on a diet deficient in vitamin A, namely, reduction in the number of small lymphocytes in the circulating blood with a corresponding rise in the percentage number of polymorpho-nuclear leucocytes.

TABLE XVIII

showing changes in weight in spleen and thymus in rats fed on a diet deficient in proteins.

No. of animal.	Weight in grs. at beginning of experiment	Weight in grs. at end of experiment.	Actual Wt of Spleen in mgs.	Wt. of spleen in mgs. per 100 grs. of original body weight.	Actual Wt. of Thymus in mgs.	Wt. of Thymus in mgs. per 100 grs. of original body weight.
61.	188.	153.	360.	191.	140.	74.
62.	174.	134	210	121.	150.	86.
63.	210.	154.	320.	152	230.	109.
64	154.	122.	200.	129.	190.	123.
65.	202	146.	280.	139	180.	89.
66.	170.	126.	210.	123.	140.	82.
67.	270.	175.	390.	144.	220.	81.
68.	145.	95.	240.	165.	180.	124.
69.	120.	75.	310.	258	190.	158.
70.	122.	110.	210.	162	190.	155.
71	150	124.	240.	160.	170.	113.
72	125.	85	260.	208.	180.	144.

As compared with those of the control animals shown in **Table** XV , the weights of the spleen and thymus are considerably reduced, thus still further tending to prove that the lymphoid tissue undergoes atrophy in any condition of chronic malnutrition.

An examination of the bone marrow of rats fed on this deficient protein ration showed in every case a considerable degree of gelatinous degeneration associated with a reduction in the number of the erythroblastic and leucoblastic cells.

DISCUSSION

Natural immunity to bacterial infection is apparently dependent on a number of factors, many of which are at present but little understood. Although certain animals are comparatively resistant to certain bacteria it is very doubtful if natural immunity is ever entirely absolute. By some means it is usually possible to break down the natural immunity of an animal to a given bacterial species, and by studying the method by which the rupture is made valuable light is thrown on the defence mechanism of the body.

In dealing with immunity it is usual to regard the body as possessing two methods of defence; the one, due/

due to the bactericidal powers of the serum, is manifested by the occurrence of such phenomina as bacteriolysis, agglutination, precipitin formation, and opsonic activity and antitoxin formation: the other is due to the phagocytic action of certain of the body cells. Although the actual source of bacteriolysins, agglutinins, etc., is not known, there is some evidence to show that the polymorpho-nuclear leucocytes play a part in their production. In the diseases due to lack of vitamins, the evidence at present tends to show that there is no decrease in the power of agglutin, complement or immune body formation nor in the opsonic activity of the serum. This evidence must be correlated with the fact that there is no marked diminution in the number of polymorph-nuclear leucocytes in the peripheral blood stream as the result of deficiencies of vitamins A, B and C.

In guinea-pigs suffering from chronic scurvy there is no failure to destroy moderate doses of infecting organisms: it is only with somewhat larger doses that the scorbutic animal is unable to deal. In other words, the scorbutic animal has less reserve to draw upon in combating infection. Attention is, therefore, naturally directed to the source of the neutrophil leucocytes — in the bone marrow. Here degenerative/

degenerative changes were found to a greater or less extent in all the deficiency diseases. In scurvy, there was found in the acute stage marked congestion and haemorrhage into the bone marrow, while in the chronic stage the congestion had given way to fibrous and gelatinous degeneration. It is of interest to note that Firket (1921[7]) has recently shown that a precisely similar pathological picture is produced in the bone marrow of rabbits by injections of saponin. A few days after the infection there is found intense congestion in the capillaries of the bone marrow. Haemorrhages occur and in a short time are replaced by a gelatinous fibrous tissue in which are left only a few myelocyte cells.

In deficiencies due to vitamins A ~~and~~, B and C there are found in the bone marrow areas of gelatinous degeneration which in some cases are very extensive. similar gelatinous degeneration also occurs as the result of diets deficient in protein and, as was shown by Jackson (1904[57]), as the result of complete starvation.

Degenerative changes in the bone marrow have for long been associated with a diminished power of resistance to bacterial infection. Although the exact method by which the pneumococcus is destroyed in/

in the human body is unknown, it is well known that a feeble leucoblastic reaction is of grave prognosis. Such a feeble leucoblastic reaction occurs in those whose bone marrow has undergone fatty degeneration as the result of the long continued abuse of alcohol.

More recently, however, it has been possible to prove experimentally that an aplastic condition of the bone marrow is associated with a reduced resistance to disease. Selling (1911[79]) first showed that benzol had a selective action on the haemopoietic cells of the rabbit's bone marrow, producing in a few days an aplastic condition. Such rabbits thus rendered aplastic by benzol were shown by Winternitz and Kline (1915[41]) to be more susceptible to pneumococcal infection than healthy control rabbits. Weiskoten and his colleagues (1920[80]) have also noted that rabbits exposed to benzene vapour were far more susceptible to bacterial infection than controls. As bearing on the role of the polymorpho-nuclear leucocyte in the formation of antibodies, it is of interest to note that Hektoen (1916[81]) found a decreased production of antibodies in rabbits rendered aplastic with benzol, while Ruskø (1914[82]) had shown that benzol-intoxicated animals produced haemalysins and precipitins less efficiently than normal animals.

In/

In determining the resistance of an animal to bacterial infection, the condition of the bone marrow is an important factor which must thus be taken into consideration.

Other factors , doubtless, also play a part in reducing the resistance of an animal which has been fed on a deficient diet. The extensive fatty degeneration of the myocardium found in guinea-pigs with chronic scurvy ,inoculated with pneumococcus, suggests that the heart muscle of the scorbutic animal may be especially susceptible to the action of toxins. In the case of animals fed on a deficiency of vitamin B, the lowering of the body temperature appears to be a factor in decreasing the natural immunity to certain organisms. Finally, in the case of animals lacking vitamin A there is a curious local reduction in the resistance of certain cells, noticeably those of the conjunctiva and possibly also those of the terminal bronchioles.

In the case of the deficiency diseases and bacterial infection, Bordet (1920) would, in part at least, appear to be correct when he states that "the fate of the infected organism depends essentially on the efficacy of its phagocytic defence". Other factors, however, play their part in reducing the resistance/

resistance of the animal fed on a deficient diet. Much patient research is yet needed before the whole of the drama stands revealed. As Gowland Hopkins aptly says "In a country rich in gold observant wayfarers may find nuggets on their path, but only systematic mining can provide the currency of nations". In the case of the vitamins, systematic mining has as yet but just begun.

CONCLUSIONS.

VITAMIN C.

(1) There is no marked reduction in the cellular elements of the blood in experimental scurvy, either acute or chronic.

(2) The bone marrow in acute scurvy shows congestion and haemorrhage: in the chronic stage there is fibrous and gelatinous degeneration.

(3) The opsonic power of the serum is not reduced in chronic scurvy.

(4) Guinea-pigs suffering from chronic scurvy show a slightly reduced resistance to bacterial infection, as exemplified by pneumococcus, staphylococcus, streptococcus and bacillus coli.

VITAMIN B./

VITAMIN B.

(1) In rats and pigeons fed on a diet deficient in vitamin B, there is a slight reduction in the number of red cells in the blood.

(2) In rats there is a leucopenia, affecting chiefly the lymphocytes.

(3) In rats there is marked atrophy of the lymphoid tissue.

(4) Both rats and pigeons show changes in the bone marrow.

(5) Pigeons are rendered susceptible to infection with the pneumococcus by a diet of polished rice.

VITAMIN A.

(1) Rats fed on a diet lacking in vitamin A show no marked reduction in the red cells.

(2) There is, however, a leucopenia, affecting chiefly the lymphocytes.

(3) Rats show an atrophy of the lymphoid tissue.

(4) In some cases there is a gelatinous change in the bone marrow.

(5) The relationship of keratomalacia and broncho-pneumonia to a deficiency of vitamin A is discussed.

DIETS/

DIETS DEFICIENT IN PROTEIN.

(1) Rats fed on deficient protein diet show no anaemia, but a slight leucopenia.

(2) There is atrophy of the spleen and thymus.

(3) The bone marrow undergoes gelatinous degeneration.

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CHART III.

Pigeons injected with 0·45 gr pyramidon:
Control:- o----o---. pigeon injected with pneumococci:- ●—●—

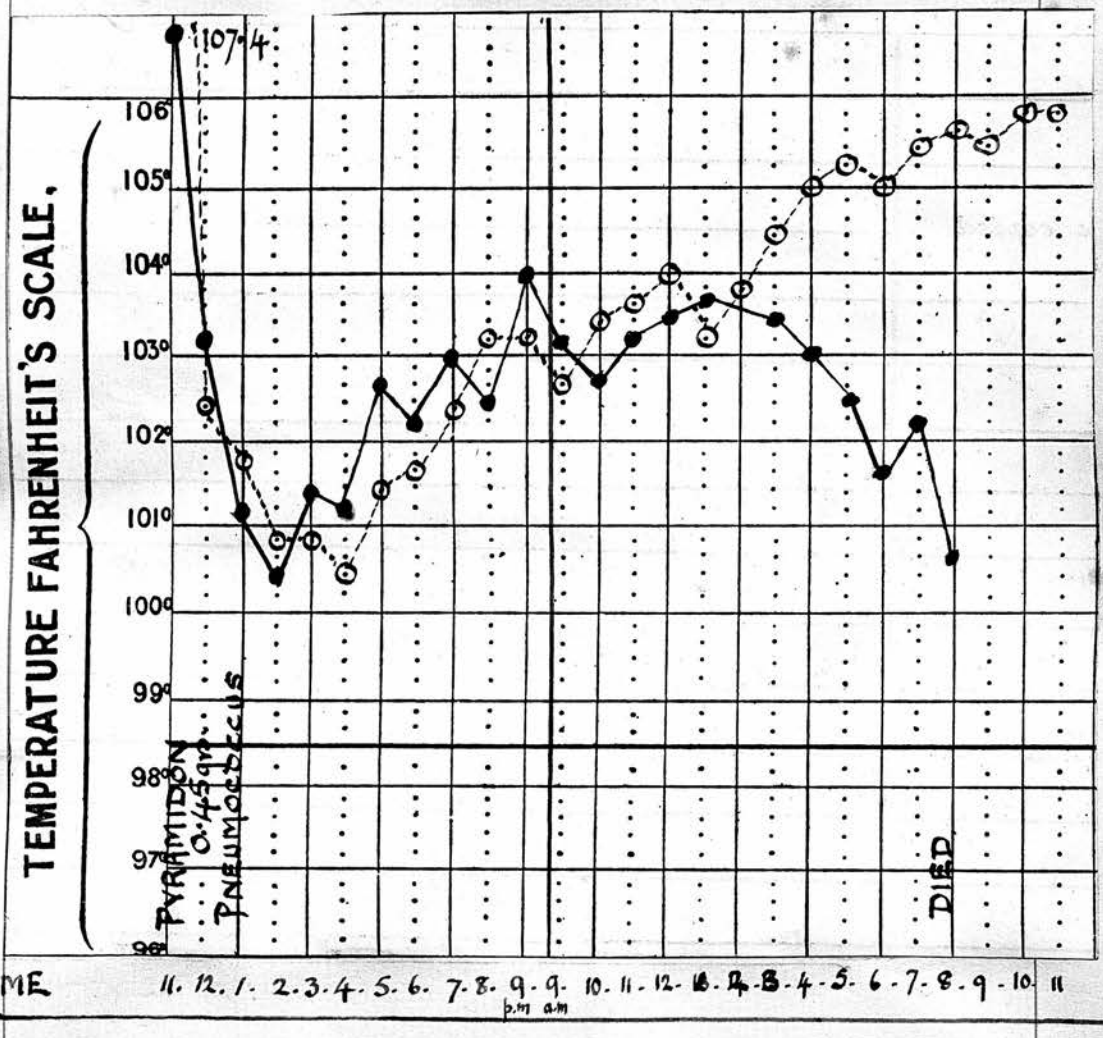


CHART IV

Pigeon fed on polished rice for 22 days, & inoculated intraperitoneally with pneumococcus

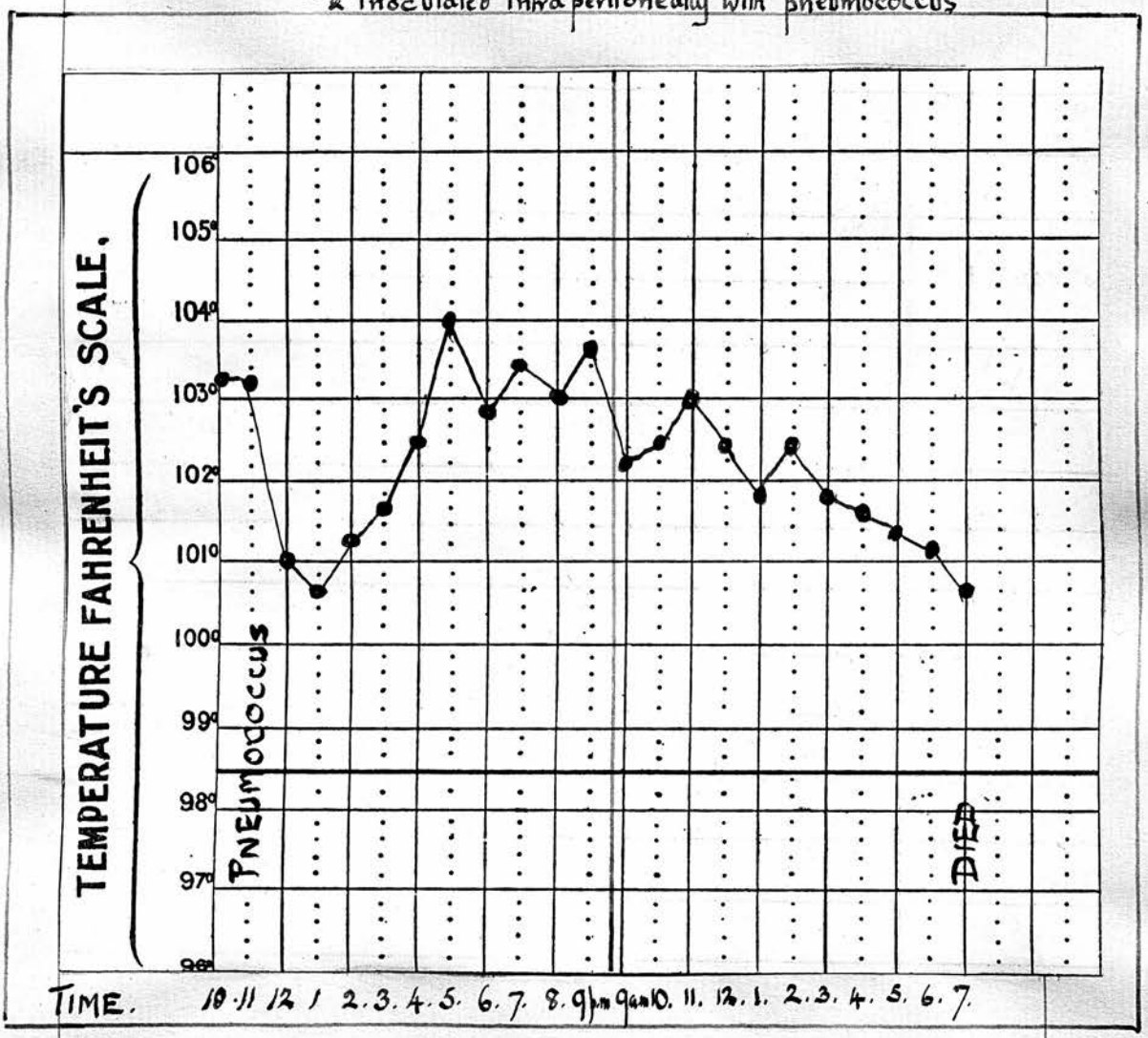


CHART V.

Pigeon fed on polished rice for 24 days and inoculated intraperitoneally with pneumococci.

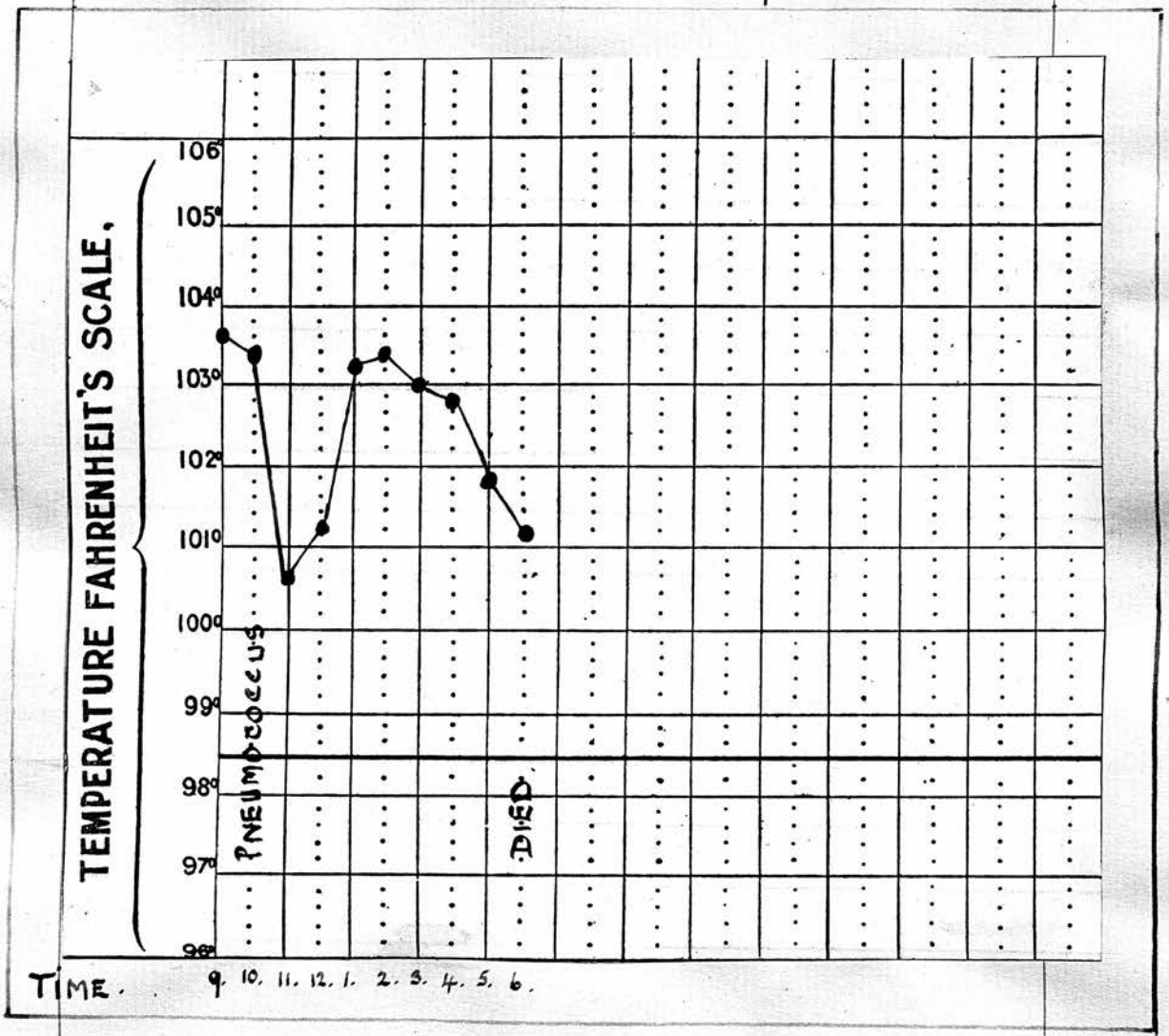


CHART VI

Pigeon fed on polished rice for 24 days
and inoculated intraperitoneally with pneumococci.

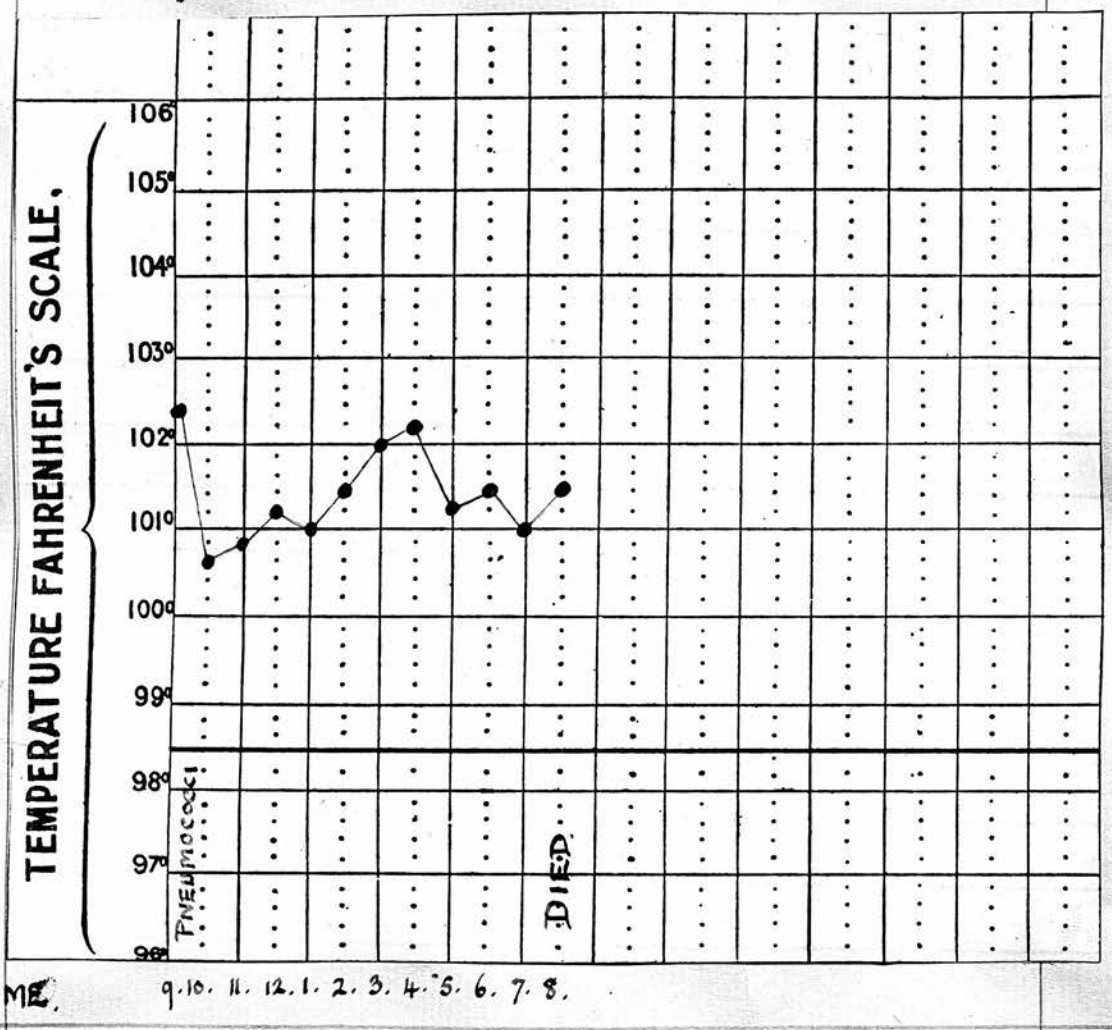


CHART VII

Pigeon fed on polished rice for 23 days and inoculated intraperitoneally with pneumococci

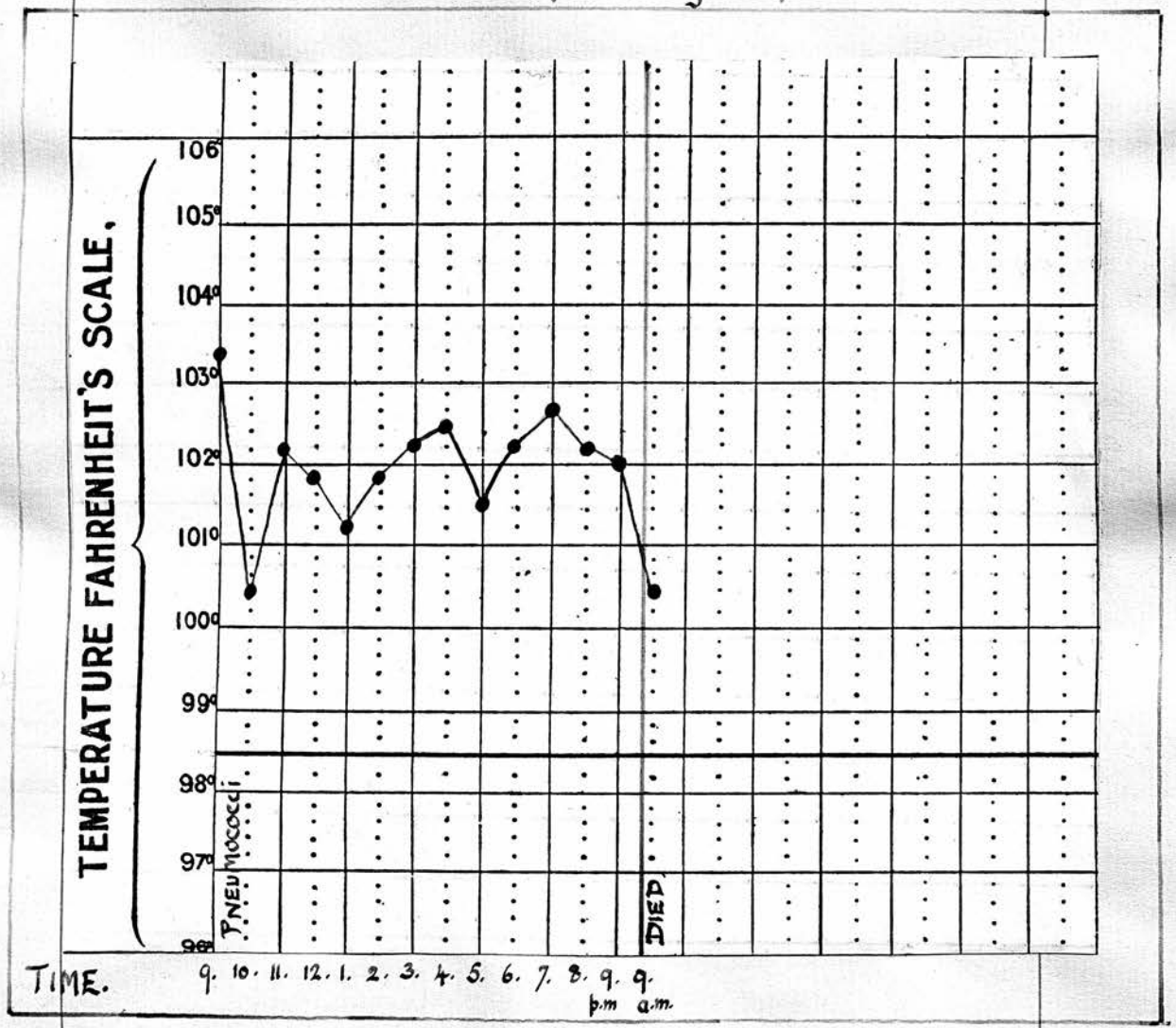


PLATE I.

Fig. 1.

Fig. 2.

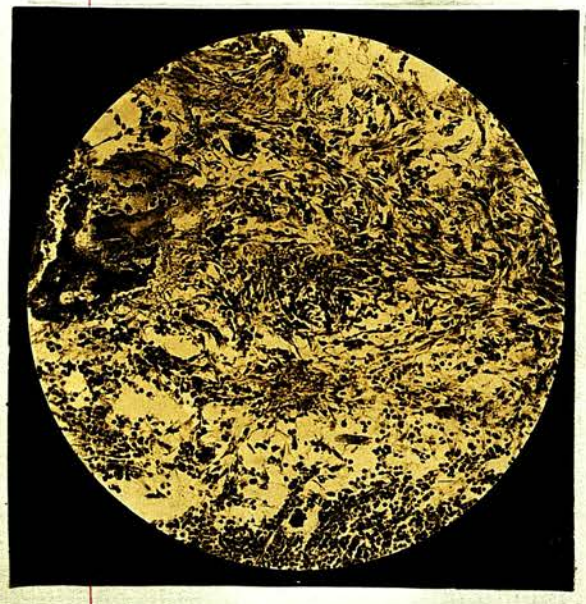
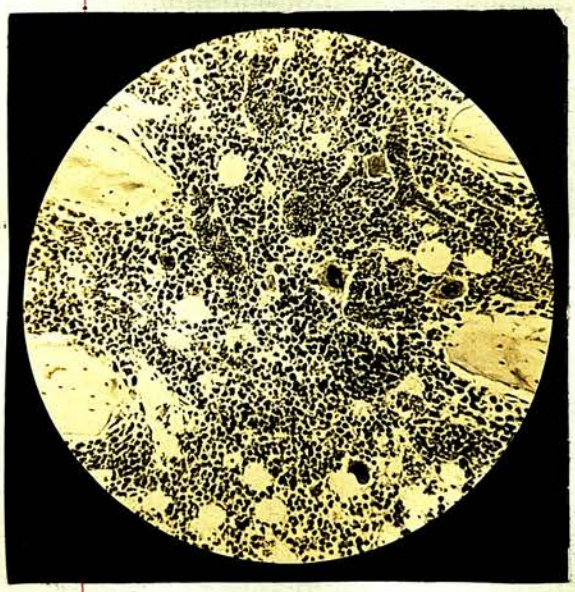


Fig. 3.

Fig. 4.

PLATE II.



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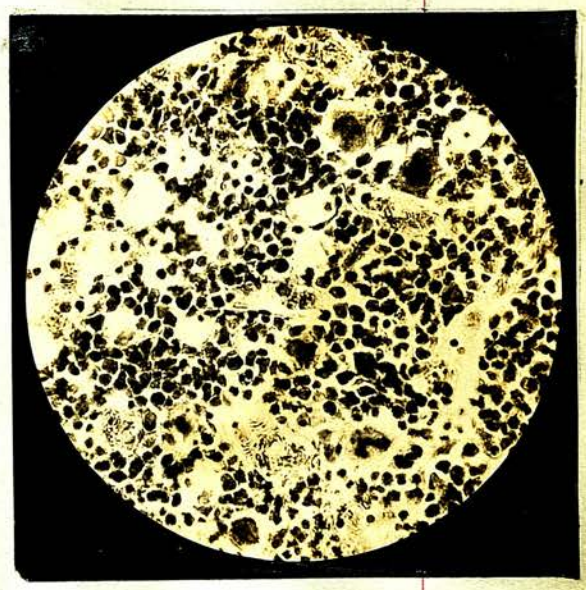

Fig. 6.

Fig. 7.

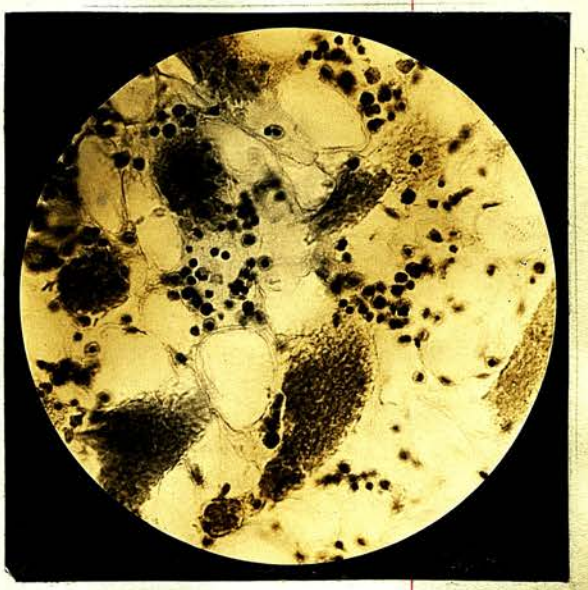

Fig. 8

PLATE III

Fig. 9.

Fig. 10.

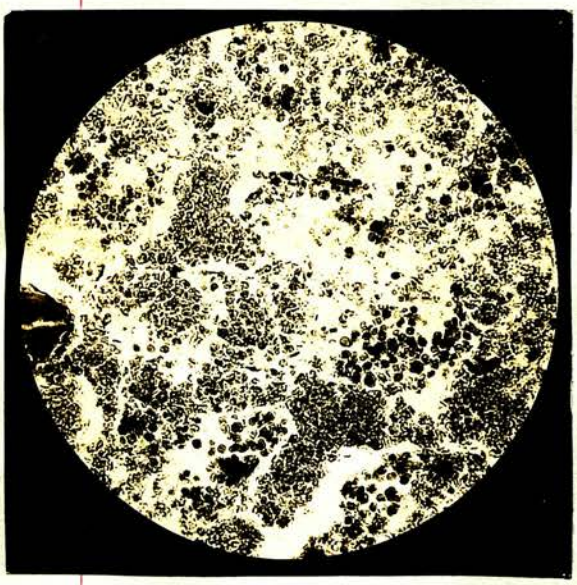

Fig. 11.

Fig. 12.

PLATE IV.

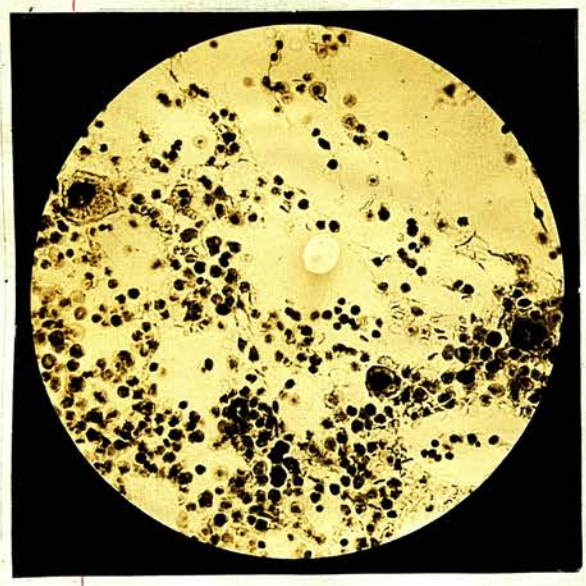

Fig. 13.

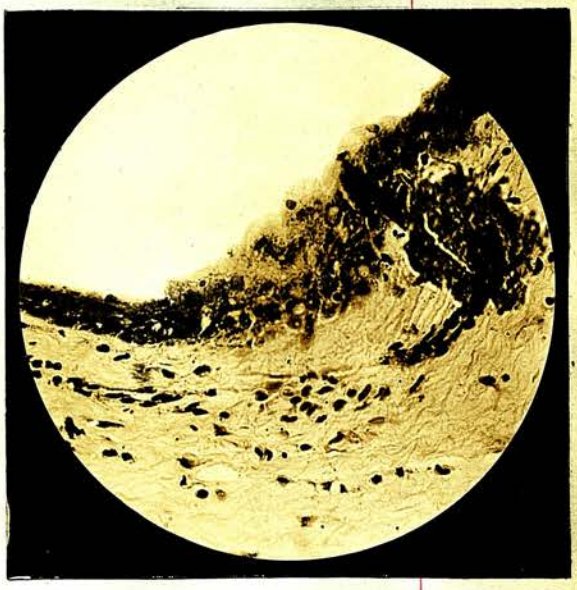

Fig. 14.

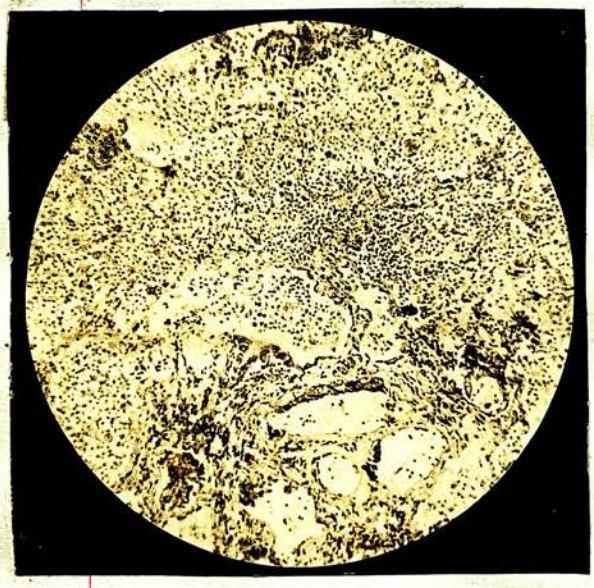

Fig. 15.

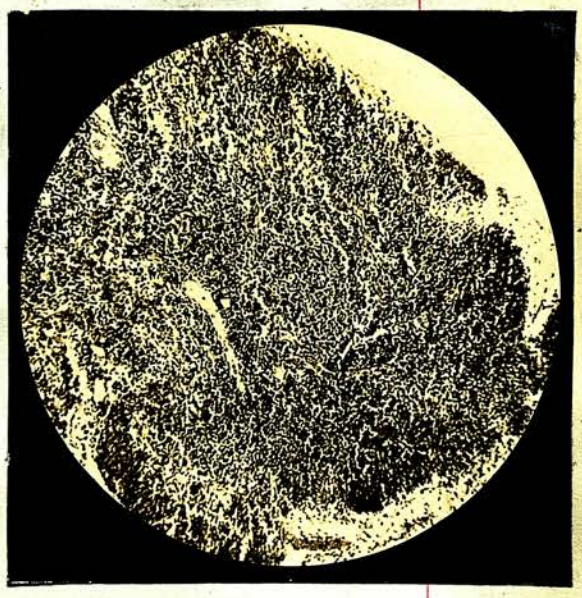

Fig. 16.

DESCRIPTION OF PLATES.

Plate I.

Fig. 1. Bone marrow of normal guinea-pig, showing proportion of fat and haemopoietic cells. Haemat. and Eos. C.B. x 80.

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

Fig. 3. Bone marrow of guinea-pig with chronic scurvy. Fibrosis. Haemat. and Eos. C.B. x 80

Fig. 4. Bone marrow of guinea-pig with chronic scurvy. Gelatinous degeneration. Haemat.and Eos. C. B. x 80.

Plate II.

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

Fig. 6. Ditto. x 250.

Fig. 7. Bone marrow of guinea-pig with chronic scurvy and pneumococcal infection: absence of leucoblastic reaction. Haemat. and Eos. C.B. x 80.

Fig. 8. Ditto. x 250.

Plate III.

Fig. 9. Bone marrow of guinea-pig with chronic scurvy and bacillus coli infection: absence of leucoblastic reaction. Haemat. and Eos. C.B. x 80.

Fig. 10. Bone marrow of normal rat. Haemat. and Eos. C. B. x 250.

Fig. 11. Bone marrow of rat fed on diet lacking in vitamin B: congestion: compare with Fig. 2. Haemat. and Eos. C.B.

Fig. 12. Heart blood of pigeon fed on diet lacking in vitamin B: pneumococcal septicaemia. Thionin blue. x 1000.

Plate IV.

Fig. 13. Bone marrow of rat fed on diet lacking in vitamin A: area of gelatinous degeneration. Haemat. Eos. C.B. x 250.

Fig. 14. Cornea of rat fed on diet lacking vitamin A. Keratomalacia. Haemat. and Eos. C.B. x 250.

Fig. 15. Lung of rat fed on diet lacking vitamin A. Broncho-pneumonia. Haemat. and Eosin C.B. x 80

Fig. 16. Thymus of rat fed on diet lacking vitamin A, Commencing atrophy of lymphoid tissue. Haemat. and Eos. C.B. x 80.