IX. THE BACTERICIDAL ACTION OF THE BLOOD IN CERTAIN DIETARY DEFICIENCIES.

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IN the present paper an attempt has been made to determine the effects of diets deficient in vitamins and in certain other essential factors on the bactericidal power of whole blood.

Zilva [1919] has shown that the power to form agglutinins, complement and immune body is not decreased in the rat as the result of diets lacking in vitamins, while Werkman [1923] has found that both in the rat and rabbit there is no inability to form agglutinins, precipitins, haemolysins and bacteriolysins on diets deficient in vitamins A and B. Findlay and Mackenzie [1922], confirmed by Werkman [1923], could find no defect in the opsonic power of the blood. Smith and Wason [1923] however stated that the bacteriotropic and bactericidal action of the serum on *B. typhosus* was reduced in rats fed on diets lacking vitamins A and B. In the case of guinea-pigs fed on a diet deficient in vitamin C, Findlay [1923] noted a diminished leucocytic response to bacterial infection, while in the case of pigeons lacking vitamin B the peritoneal exudate had less bactericidal power than that of control birds.

The present investigations have been carried out on the bactericidal power of the whole blood for *Staphylococcus aureus* in normal rats and in rats subjected to the following procedures:

- A. Fed on a diet lacking vitamin A and the antirachitic factor.
- B. Fed on a diet lacking vitamin A and the antirachitic factor and exposed to ultra-violet light.
- C. Fed on a diet lacking the antirachitic factor and phosphorus.
- D. Fed on a diet lacking vitamin B.

The rats used in these experiments were all bred from laboratory stock. As preliminary experiments had shown that the employment of anaesthetics in killing the animals caused variations in the bactericidal power of the blood it was decided to kill the rats by a blow on the back of the head; the thorax was then opened and the blood was removed from the heart with aseptic precautions. The rats used as controls were fed on laboratory diet with the addition of cod-liver oil and marmite. All the animal experiments were carried out by one of us (G. M. F.). The observations on the bactericidal power of the blood were made by the slide cell method of Wright, Colebrook and Storer [1923].

A. RATS FED ON A DIET DEFICIENT IN VITAMIN A AND THE
ANTIRACHITIC FACTOR.

The rats used in this experiment were placed on the following diet:

Caseinogen	...	...	14 g.		
Starch	...	...	77		
Marmite...	...	...	5		
Inorganic salt mixture:					
NaCl	...	...	0.32 g.	CaH ₄ (PO ₄) ₂ .2H ₂ O	1.02 g.
MgSO ₄	...	...	0.56	Ca lactate	2.46
NaH ₂ PO ₄ .2H ₂ O			0.66	Fe citrate	0.22
K ₂ HPO ₄	...		1.76		

Though lacking both vitamin A and the antirachitic factor, animals placed on this diet do not develop rickets since the proportions of calcium and phosphorus are correct.

In the first experiment the rats were allowed to continue on the diet until they began to show symptoms due to a deficiency of vitamin A. The body temperature at the time of death and the bactericidal power of the blood are shown in Table I.

It will be seen that the body temperature of the normal rat varies from about 37.0° to 38.0°, while the percentage of staphylococci killed by the blood is as a rule about 70. There is thus a definite fall in the bactericidal power of the blood in association with a deficiency of vitamin A and the antirachitic factor, but only after the onset of keratomalacia or bronchopneumonia.

In the second experiment young rats were placed on the diet deficient in vitamin A and the antirachitic factor; they were then killed after varying periods on the diet, a random selection being made. Here again, as shown in Table II, there is no fall of bactericidal power until after the onset of keratomalacia.

B. RATS FED ON A DIET DEFICIENT IN VITAMIN A AND THE ANTIRACHITIC
FACTOR, BUT EXPOSED TO ULTRA-VIOLET LIGHT.

The rats were placed on the same diet as in the previous experiments but were irradiated for 20 minutes each day with a mercury vapour lamp at a distance of 8 inches.

The experiments of Colebrook, Eidenow and Hill [1924] have shown that when a healthy animal is exposed to ultra-violet light there results an increase in the bactericidal power of the blood: this increase lasts for at least 2 hours after the exposure but tends to disappear 4 hours after exposure. The rats were killed after varying periods on the diet and, except in two cases, from 6 to 24 hours after exposure to the ultra-violet light. It will be seen from Table II that the bactericidal power of the blood of the animals exposed to

light did not fall in the same way as in those kept in diffused daylight; this must be correlated with the fact that the animals exposed to ultra-violet light did not develop keratomalacia or other intercurrent infections so rapidly as the non-exposed rats. The growth curves also of the irradiated rats were better than those of the non-exposed rats, a confirmation of the findings of Hume [1922] and Goldblatt and Soames [1922].

Table I. *The Bactericidal Power of the Blood in Control Rats and in Rats on a Diet deficient both in Vitamin A and the Antirachitic Factor.*

No. of rat	Body temperature at death	Percentage of staphylococci killed	Remarks
<i>Vitamin-rich diet.</i>			
1	37.7	72	—
2	37.1	65	—
3	37.4	67	—
4	37.3	74	—
5	37.2	73	—
6	37.6	76	—
7	37.5	68	—
Average	37.4	71	—
<i>— Vitamin A and — Antirachitic Factor.</i>			
8	37.2	67	— Keratomalacia
9	37.2	46	+ „
10	37.7	60	— „
11	37.2	71	— „
12	36.9	50	{ — „
13	37.6	65	{ + Broncho-pneumonia
14	36.5	43	+ Keratomalacia
15	37.1	38	+ „
16	37.2	53	+ „
17	37.1	45	+ „
18	37.2	55	+ „
19	37.5	57	{ — „
			{ + Broncho-pneumonia
Average	37.2	54	—
<i>— Vitamin A and — Antirachitic factor. Single exposure to Ultra-violet Light 1 hour before death.</i>			
20	37.2	48	+ Keratomalacia
21	35.0	47	+ „
22	37.1	45	+ „
23	37.2	54	+ „
Average	36.6	48.5	—

Four rats suffering from keratomalacia were exposed to ultra-violet light for 30 minutes and the bactericidal action of the blood tested 1 hour later. As shown in Table I the bactericidal action of the blood in these animals was still low after irradiation. This is in agreement with the observations of Colebrook [1924], who found that patients with a low bactericidal power of the blood do not show any increased bactericidal action of the blood after exposure to ultra-violet light.

Table II. *The Bactericidal Power of the Blood of Rats on a Diet deficient both in Vitamin A and the Antirachitic Factor and killed after varying periods on the Diet.*

No. of rat	No. of days on diet	Body temperature at death	Percentage of staphylococci killed	Remarks
<i>Vitamin-rich diet.</i>				
24	—	37.4	78	—
25	—	37.6	79	—
26	—	37.8	76	—
27	—	37.2	76	—
28	—	37.1	67	—
29	—	38.0	70	—
30	—	37.4	75	—
Average	—	37.5	74	—
<i>— Vitamin A and — Antirachitic factor.</i>				
31	7	37.6	75	— Keratomalacia
32	7	37.9	78	— "
33	7	37.7	77	— "
34	33	36.7	43	+ "
35	33	35.4	38	+ "
36	54	36.7	47	+ "
37	54	36.8	45	+ "
Average	—	37.0	58	—
<i>— Vitamin A and — Antirachitic factor. Daily exposure to Ultra-violet Light.</i>				
38	7	37.2	78	— Keratomalacia
39	7	37.4	77	— "
40	7	37.6	76	— "
41	26	37.3	90	— " } killed 1 hour after exposure to ultra-violet light
42	26	37.0	92	— " }
43	33	37.8	75	— "
44	33	38.0	71	— "
45	54	37.7	69	— "
46	54	38.4	72	— "
47	78	38.0	72	— "
48	78	38.0	69	— "
49	92	36.9	54	+ "
Average	—	37.7	74	—

C. RATS FED ON A DIET LACKING THE ANTIRACHITIC FACTOR AND PHOSPHORUS.

When young rats are placed on the following diet [Pappenheimer, McCann and Zucker 1922]:

White flour	...	...	80.9 g.		
Egg albumin	...	...	10.0		
Butter fat	...	...	5.0		
Salt mixture:					
KCl	...	...	0.850 g.	Ca lactate	... 2.000 g.
Na ₂ CO ₃	...	...	0.850	Fe citrate	... 0.100
MgCO ₃	...	...	0.286	KI	... 0.0002

they invariably develop rickets. The diet is adequate for full growth since it contains both vitamins A and B; it can be rendered antirachitic by the

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addition of cod-liver oil or phosphates or by exposing the animals to ultra-violet light. The rats in this experiment were allowed to remain on the diet for 3 weeks before testing the bactericidal power of the blood. All the rats had well-developed rickets. Reference to Table III shows that in two cases the bactericidal power of the blood was normal while in four cases it was greatly reduced.

Table III. *The Bactericidal Power of the Blood of Rats on a Diet deficient in the Antirachitic Factor and Phosphorus.*

No. of rat	No. of days on diet	Body temperature at death	Percentage of staphylococci killed
<i>Vitamin-rich diet.</i>			
50	—	37.4	68
51	—	37.2	74
52	—	37.6	76
53	—	37.8	67
Average	—	37.5	71
<i>Rickets Rats.</i>			
54	21	36.9	30
55	21	37.2	20
56	21	37.2	48
57	21	37.5	74
58	21	36.8	70
59	21	36.8	30
Average	—	37.1	45

Table IV. *The Bactericidal Power of the Blood of Rats on a Diet deficient in Vitamin B.*

No. of rat	No. of days on diet	Body temperature at death	Percentage of staphylococci killed
<i>Vitamin-rich diet.</i>			
60	—	37.7	75
61	—	37.1	65
62	—	37.1	67
63	—	37.3	72
64	—	37.2	73
Average	—	37.3	70
<i>— Vitamin B.</i>			
65	25	35.6	42
66	25	35.4	50
67	32	35.1	38
68	32	35.1	33
69	35	< 35.0	38
70	35	< 35.0	39
71	39	35.2	50
72	39	< 35.0	50
73	41	36.2	51
Average	34	35.3	43

D. RATS FED ON A DIET LACKING VITAMIN B.

In this experiment the rats were fed on the following diet:

Caseinogen	...	...	14 g.			
Starch	...	...	77			
Cod-liver oil	...	...	5			
Inorganic salt mixture:						
NaCl	...	...	0.32 g.	CaH ₄ (PO ₄) ₂ .2H ₂ O	1.02 g.	
MgSO ₄	...	...	0.56	Ca lactate	...	2.46
NaH ₂ PO ₄ .2H ₂ O			0.66	Fe citrate	...	0.22
K ₂ HPO ₄	...		1.76			

The length of time on the diet, the body temperature and the bactericidal action of the blood are shown in Table IV. A fall in the bactericidal action of the blood occurs as the result of a diet deficient in vitamin B, apart from the occurrence of any bacterial infection, though the lowering of the bactericidal power in this type of deficiency is always associated with a fall in body temperature. In the case of animals fed on diets deficient in vitamin B, this decline in body temperature takes place some days before death.

DISCUSSION.

The question naturally arises as to whether the bactericidal power of whole blood is due to the action of the leucocytes or the serum. Smith and Wason [1923], as previously indicated, found that the bactericidal power of the serum against *B. typhosus* was decreased as the result of diets deficient in vitamins A and B. It is doubtful whether any great significance can be attached to their results, as the bactericidal action on *B. typhosus* of the serum of normal rats is known to vary very greatly. The blood of wild and black rats as a rule has no bactericidal action on *B. typhosus*, while that of white rats has a slight bactericidal action.

The serum of the rat has little or no bactericidal action on *Staphylococcus aureus*.

The work of Wright, Colebrook and Storer [1923], on the other hand, has proved that the bactericidal power of whole blood against *Staphylococcus* is largely due to the polymorphonuclear leucocytes.

Any factor which tends either to reduce the numbers of the polymorphonuclear leucocytes or to impair their functional activity might therefore decrease the bactericidal power of the blood. It is thus of interest to determine whether there is any reduction in the number of the polymorphonuclear leucocytes in the blood in association with vitamin deficiencies.

Total and differential white cell counts were made in 10 normal rats and in 10 rats with keratomalacia as the result of a diet lacking vitamin A. The following figures were obtained:

Control rats:	9450 leucocytes per mm. ³	Polymorphs	35 %
— Vitamin A rats:	8170	„ „ „ „	48 „

There is thus little difference in the total leucocyte counts, while in the case of the vitamin A deficient rats there is a polymorphonuclear leucocytosis, probably as a result of the bacterial infection. Cramer, Mottram and Drew [1921] have obtained similar figures. When rats are fed on diets deficient in vitamin B, there is a fall in the total leucocyte count, but this fall only affects the lymphocytes, the polymorphs, as shown by Cramer, Mottram and Drew [1921] exhibiting little or no decrease in number. It is obvious therefore that the fall in bactericidal power of the blood in vitamin A and B deficiencies cannot be due to a decrease in the number of polymorphonuclear leucocytes.

There is, however, a possibility that the killing power of all the polymorphonuclear leucocytes in the blood is not equal. If young and aged polymorphonuclear leucocytes are of less vigour than adult cells, then the bactericidal power of the blood should tend to decrease whenever there is an excessive proportion of very young or very old polymorphs in the blood stream, unaccompanied by a definite leucocytosis. An excessive number of aged polymorphonuclear leucocytes for instance would survive in the blood stream in a condition where there is diminished leucoblastic activity of the bone marrow unassociated with bacterial infection. Such a condition is found in animals fed on diets deficient in vitamin B, where the bone marrow, as shown by Shipley, McCollum and Simmonds [1921], exhibits numerous small haemorrhages. In addition Happ [1922] has found that in rats suffering from a deficiency of vitamin B there is a very high proportion of aged or moribund polymorphs in the blood stream.

CONCLUSIONS.

1. The blood of normal rats, tested against *Staphylococcus aureus*, shows a killing power of about 70 %.
2. Rats fed on a diet deficient in vitamin A and the antirachitic factor show a reduction in the bactericidal power of the blood only after the onset of keratomalacia or some other infection.
3. In rats fed on a diet deficient in vitamin A and the antirachitic factor, but exposed to ultra-violet light, the onset of bacterial infection and the reduction in the bactericidal power of the blood are delayed.
4. Rats suffering from keratomalacia as the result of a diet deficient in vitamin A continue to show a low bactericidal power of the blood after exposure to ultra-violet light.
5. Rats fed on a diet lacking phosphorus and the antirachitic factor show, in most cases, a reduction of the bactericidal power of the blood.
6. Rats fed on a diet lacking vitamin B show a reduction in the bactericidal power of the blood unassociated with bacterial infection but possibly correlated with the fall in body temperature.

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