LVI. OPSONINS AND DIETS DEFICIENT IN VITAMINS.

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ALTHOUGH it is well known that animals fed on diets deficient in vitamins frequently die from intercurrent bacterial infections, the reason for this apparent failure in the protective mechanism of the body is at present unknown. Hektoen [1914] found that the serum reactions were quite normal in animals fed for considerable periods on artificial diets, while later, Zilva [1919] could not determine any decrease in the power to form agglutinins, complement and immune body in animals fed on diets deficient in vitamins.

In the present investigation the opsonic activities of the serum were studied in animals fed on diets deficient in vitamins.

DIETS DEFICIENT IN VITAMIN A.

Rats were employed for this experiment. Four partially grown rats varying from 106 to 120 g. were placed on a diet consisting of caseinogen, starch, cotton seed oil, marmite and an inorganic salt mixture. After ten weeks the animals were killed. One of the animals was suffering from keratomalacia at the time of its death. The sera from the four animals were collected in the usual manner and pooled before use.

Four normal animals fed on a diet of caseinogen, starch, cod-liver oil, marmite and inorganic salts were also killed and their sera collected to serve as a control. Leucocytes were obtained from a normal rat. The technique employed was that recommended by Wright [1921].

The opsonic activity of the two groups of sera was determined for two types of organisms, *Bacillus coli* and *Staphylococcus aureus*. One hundred polymorphonuclear leucocytes were counted on each slide. Notes were taken of (1) the number of leucocytes containing bacteria, (2) the total number of bacteria phagocytosed. The average number of bacteria per polymorphonuclear leucocyte was termed the phagocytic index. The data are recorded in Table I.

Table I.

Date of examination	Organism	Condition of animal	No. of polymorphonuclear leucocytes counted	No. of cells with bacteria	Total no. of organisms ingested	Phagocytic index
After 10 weeks on the diet	Staphylococcus	- Vitamin A	100	79	128	1.3
	<i>B. coli</i>		100	40	46	0.5
	Staphylococcus	+ Vitamin A	100	84	136	1.4
	<i>B. coli</i>		100	42	69	0.7

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

DIETS DEFICIENT IN VITAMIN B.

Rats were also used for this experiment. Six rats were fed on a diet of caseinogen, starch, cod-liver oil and an inorganic salt mixture. Three rats were killed after 21 days, the remainder after 6 weeks on the diet. Rats fed on a complete diet were used as controls. The technique employed was the same as in the first experiment. The data are given in Table II.

Table II.

Date of examination	Organism	Condition of animal	No. of polymorphonuclear leucocytes counted	No. of cells with bacteria	Total no. of organisms ingested	Phagocytic index
After 3 weeks on the diet	Staphylococcus	- Vitamin B	100	73	96	1.0
	<i>B. coli</i>		100	74	98	1.0
	Staphylococcus	+ Vitamin B	100	70	129	1.3
	<i>B. coli</i>		100	82	126	1.3
After 6 weeks on the diet	Staphylococcus	- Vitamin B	100	77	135	1.3
	<i>B. coli</i>		100	59	79	0.8
	Staphylococcus	+ Vitamin B	100	67	141	1.4
	<i>B. coli</i>		100	76	88	0.9

DIETS DEFICIENT IN VITAMIN C.

Guinea-pigs were used for this experiment. As is well known, when fed on a diet containing only small quantities of vitamin C these animals develop chronic scurvy, a condition characterised by no very definite clinical symptoms except a failure of normal growth. Six guinea-pigs, varying in weight from 250 to 300 g., were fed on an *ad libitum* diet of oats and bran, and 70 cc. of autoclaved milk a day, with the addition of 2 cc. of orange juice every third day. Six controls were placed on the same basal diet but with the addition of 10 cc. of orange juice every day. Experiments, as before, were carried out with two organisms, *Staphylococcus aureus* and *Bacillus coli*. Readings were made after

four weeks and after eight weeks on the dietary, three scorbutic and three control animals being killed on each occasion. The technique was the same as in the experiments with rats except that the leucocytes in this instance were those of a normal guinea-pig. The results are summarised in Table III.

Table III.

Date of examination	Organism	Condition of animal	No. of polymorphonuclear leucocytes counted	No. of cells with bacteria	Total no. of organisms ingested	Phagocytic index
After 4 weeks on the diet	Staphylococcus	- Vitamin C	100	94	315	3.1
	<i>B. coli</i>		100	87	115	1.1
	Staphylococcus	+ Vitamin C	100	91	330	3.3
	<i>B. coli</i>		100	84	148	1.5
After 8 weeks on the diet	Staphylococcus	- Vitamin C	100	87	261	2.6
	<i>B. coli</i>		100	82	104	1.0
	Staphylococcus	+ Vitamin C	100	92	282	2.8
	<i>B. coli</i>		100	76	95	0.9

THE PHAGOCYTIC ACTIVITY OF THE POLYMORPHONUCLEAR LEUCOCYTES IN SCURVY.

Since the opsonic activity of the serum was practically the same both in scorbutic and in control animals, an attempt was next made to determine whether the liability to infection in scurvy was in any way associated with a decreased phagocytic power of the polymorphonuclear leucocytes themselves. For this purpose two guinea-pigs of equal weight were selected, one being quite healthy, while the other had been fed on a diet deficient in vitamin C for the previous eight weeks. 1 cc. of a suspension of *S. aureus* heated for an hour at 55° and containing 200 million organisms was injected intraperitoneally into each animal. At intervals some of the peritoneal exudate was removed by means of a capillary tube; smears were made, stained with "Leishman" and examined microscopically. Two hundred polymorphonuclear leucocytes were counted on each film, a note being taken of the number of polymorphonuclear leucocytes containing 0, 1, 2, 3, and more than 3 staphylococci. The results are shown in Table IV.

Table IV.

		Percentage no. of polymorphonuclear leucocytes containing				
Time	Condition of animal	No	1	2	3	More than 3
		Staphylococci	Staphylococcus	Staphylococci	Staphylococci	Staphylococci
3 hours after injection	Scorbutic	50	27	13	8	2
	Control	44	26	16	10	4
6 hours after injection	Scorbutic	16	32	22	16	14
	Control	33	18	25	12	12
18 hours after injection	Scorbutic	36	24	26	6	8
	Control	36	18	24	16	6
30 hours after injection	Scorbutic	48	16	21	9	6
	Control	37	20	21	13	9

An examination of the pleural exudate 54 hours after the injection showed that mononuclear leucocytes were now the predominant type of cell in both animals while the polymorphonuclear leucocytes still present were undergoing degeneration.

There is no evidence to suggest any decrease in the phagocytic activity of the polymorphonuclear leucocytes as the result of a diet deficient in vitamin *C*.

In many ways the bacterial infections so frequently found in association with the deficiency diseases are analogous to the terminal infections met with in other chronic diseases. It is therefore of some interest to note that quite recently Cross [1921] has investigated the question of the opsonic index in relation to terminal infections associated with chronic bacterial 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 the disease.

As Bordet [1909] was the first to point out, the phagocytic power of the body would appear to be a relatively stable function and one not easily influenced by conditions which profoundly affect other vital activities.

CONCLUSIONS.

1. Rats fed on diets deficient in vitamins *A* and *B* do not show any decrease in the opsonic activity of the serum.
2. Guinea-pigs fed on a diet deficient in vitamin *C* do not show any decrease in the opsonic activity of the serum.
3. The phagocytic activity of the polymorphonuclear leucocytes of guinea-pigs with chronic scurvy is not decreased.

REFERENCES.

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