INFLUENCE OF DIETARY FACTORS UPON THE RESISTANCE OF THE WHITE RAT TO EXPERIMENTAL TUBERCULOSIS

I. VITAMIN A DEFICIENCY *

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A brief review of medical literature indicates that depletion of vitamin A reserve leads to a marked increase in the susceptibility of certain animals to many infections. Werkman [1] reported decreased resistance in vitamin A-deficient rats and rabbits to anthrax and pneumo-bacilli given by mouth. Lassen [2] obtained a similar result in xerophthalmic rats infected with paratyphoid bacilli by mouth and by subcutaneous injection. Boynton and Bradford [3] noted increased susceptibility to infection with bacilli of the *mucosus capsulatus* group in vitamin A-deficient animals, while in those depleted of vitamin D this was not so marked. Ackert, McIlvaine and Crawford [4] noticed a lowering in resistance of chickens inoculated with the intestinal round-worm *Ascaridia lineata,* as did McClung and Winters [5] in rats injected intraperitoneally with *Salmonella enteritidis,* when vitamin A was withdrawn from the diet of their experimental animals. Pritchett [6], on the other hand, notably enhanced the resistance of mice to infection *per os* with *B. pestis caviae,* by adding 5 per cent of butter fat or cod liver oil to their diet.

In view of these findings, we thought it would be interesting to determine to what extent vitamin A is a factor in maintaining the high resistance of albino rats to warm-blooded strains of tubercle bacilli. We chose to experiment on these rodents because of their known natural resistance to the mammalian bacilli, and because they are easily deprived of vitamin A reserve. That the albino rat is highly

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resistant to the human tubercle bacillus was demonstrated by Ornstein and Steinbach[7] in 1925. Steinbach[8] further showed that normal albino rats on a bread-and-water diet still maintain their high resistance to both human and bovine bacilli, but become more susceptible to the avian variety.

The animals used in our experiments were bred from mothers maintained on a normal diet. When 28 or 29 days old, some of them were put on a diet of 18 per cent purified casein, 4 per cent Osborne and Mendel salt mixture, 10 per cent baker's yeast and 68 per cent cornstarch, with distilled water. This ration was continued until the weight of the animals became stationary or decreased, and signs of depletion of the vitamin A reserve were noticed. Some of these depleted animals were then maintained on the vitamin A-free diet mentioned above, while others were given in addition 0.25 gm. daily of pigeon peas, which supplied a small amount of vitamin A. Controls of the same breed were kept on a full maintenance diet throughout.

The strains of tubercle bacilli employed were well known strains, all pathogenic, i. e. the human H37, bovine "Ravenel" and avian "Trudeau".

The experimental animals were divided into three main groups, with some further subdivisions. Group I was inoculated with human tubercle bacilli, Group II with bovine, and Group III with avian. The details of the experiments and results are as follows:

Group I. (Inoculated with human tubercle bacilli).

A. Seven rats, totally depleted, infected *per os,* with doses from 1 to 8 mgm. The animals lived from 15 to 33 days, an average of 23.58 days, and at autopsy showed no tuberculosis.

B. Eleven rats, totally depleted, inoculated intraperitoneally. Seven of these animals received 1 mgm. each, and the remaining four received 2, 3, 5, and 6 mgm. respectively.

The rats lived from 1 to 57 days after inoculation, an average of 22.2 days, and on pathological examination failed to reveal any evidence of tuberculosis.

C. Five rats, partially depleted, inoculated intraperitoneally with 1 mgm. of the bacilli.

These rodents lived from 21 to 42 days, an average of 37 days, after inoculation, and again these failed to disclose any evidence of macro- or microscopic tuberculosis.

D. Seven rats, on adequate diet, inoculated intraperitoneally with doses of 1 mgm.

All the animals were killed 50 days after inoculation, and the results were entirely negative for tuberculosis.

Group II. (Inoculated with bovine tubercle bacilli).

A. Four rats, totally depleted, inoculated intraperitoneally with doses of 1 mgm.

The animals lived from 6 to 12 days after inoculation, an average of 8.2 days. No tuberculosis developed within that period of time.

B. Six rats, partially depleted, inoculated intraperitoneally with 1 mgm. doses. The duration of life was from 14 to 31 days after inoculation, an average of 20 days. Pathological studies failed to reveal evidences of tuberculosis.

C. Six rats on adequate diet, inoculated intraperitoneally with 1 mgm. doses. All the animals were killed 49 days after inoculation, and no evidences of tuberculosis were found at autopsy.

Group III. (Inoculated with avian tubercle bacilli).

A. Eight rats, totally depleted, inoculated intraperitoneally with 1 mgm. doses.

The rats lived from 10 to 33 days after inoculation, an average of 19.2 days. Of the 8 animals, 4, or 50 per cent, presented definite macro- and microscopic evidence of tuberculosis (see Table 1.), and 3 showed caseous changes in the lesions.

B. Eight rats, partially depleted, inoculated intraperitoneally with 1 mgm. doses.

One animal died 34 days after inoculation, while the remaining 7 were killed from 54 to 56 days after inoculation. Tuberculosis of the peritoneum was found in 4, or 50 per cent (see Table II).

C. Eight rats on adequate diet, inoculated intraperitoneally with doses of 1 mgm.

The rats were killed and autopsied 49 days after inoculation (see Table III), and again 50 per cent of the animals on autopsy studies revealed macro- and microscopic tuberculosis.

Group IV. (Controls.)

Five rats, totally depleted, *not* inoculated, studied to determine length of life in depleted rats. They lived from 6 to 24 days, an average of 16.2 days.

MORBID ANATOMY

Lesions of tuberculosis were found only in animals inoculated with avian tubercle bacilli (Group III, A, B and C).

Group III A was composed of eight totally depleted rats, of which four presented tuberculous changes at autopsy. These changes consisted in the formation of small, discrete tubercles that projected from the peritoneal surface of the spleen, liver or intestines. Central necrosis and caseation were not noted in the tubercles so situated. In all of the affected animals a fairly large mass composed of tubercles was found in the mesentery, a large central area of caseous necrosis having developed in its center in one of the rats. In

two other animals early foci of necrosis were observed in the mesenteric mass, but no widespread caseation.

Group III B included eight partially depleted rats, of which four contracted tuberculosis. Small, discrete and non-caseating tubercles were found in the peritoneum over the spleen of three animals. The fourth rat had one small mass at the hilus of the spleen, composed of conglomerate, non-caseating tubercles. In one of the infected rats there were larger conglomerate tubercles in the omentum and mesentery in addition to those on the spleen, but these did not form the relatively large masses found in the animals of Group III A.

Group III C comprised eight rats fed on an adequate diet. Four animals became infected with tuberculosis. In two of these the only evidence was in the partial replacement of a moderately enlarged abdominal lymph gland by groups of epithelioid cells. In a third animal, discrete tubercles were found over the spleen. In the fourth infected rat there was a large area of reaction with epithelioid cells in the sub-peritoneal layer of the anterior abdominal wall, with partial replacement of the musculature by these cells. This lesion may have marked the point of inoculation. Calcium had been deposited in parts of this area and in the affected abdominal lymph glands of one of the animals in this group.

DISCUSSION

No conclusions can be drawn from the negative result obtained in the totally depleted rats that were inoculated intraperitoneally with bovine tubercle bacilli (Group II A.), since they lived for an average of only 8.2 days after inoculation. These experiments demonstrate nevertheless the high resistance of the white rat to infection with human and bovine tubercle bacilli, and substantiate the observations of other investigators. This natural protection apparently is not broken down by the absence of vitamin A in the diet.

As regards infection with the avian organism it was found that 50 per cent of the animals developed tuberculosis irrespective of whether they were kept on an adequate or deficient diet. This percentage is in keeping with previous findings, such as those of Steinbach. It is interesting to note that the tuberculous changes which occurred in this group of animals were limited exclusively to the peritoneum and abdominal lymph glands.

Although deprivation of vitamin A had no demonstrable influence on the incidence of infection in rats inoculated with human, bovine or avian tubercle bacilli, it should be pointed out that there were some differences in the pathological character of the tuberculous process induced by infection by the avian organisms in totally depleted animals. The course of the disease under such conditions was accelerated, the areas of tuberculous change not only being definitely larger, but caseation being also present (see Table I, Group III A, Rats No. 5, 6 and 8).

SUMMARY

1. White rats maintained either on adequate diets, or on diets partially or totally deficient in vitamin A, were inoculated with human, bovine or avian strains of tubercle bacilli, by feeding or by intraperitoneal injection.

2. All rats inoculated with human and bovine organisms failed to develop tuberculosis, irrespective of the diet on which they were kept, and of the method of inoculation.

3. Fifty per cent of all rats (even when on normal diet) which were inoculated with the avian strain showed tuberculosis of the peritoneum and abdominal lymph glands at autopsy.

4. The evolution of the avian tuberculous process seemed to be accelerated in the totally depleted rats as compared with those maintained on a partially deficient or adequate diet. Furthermore, caseation was noted only in animals totally depleted of vitamin A.

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TABLE I

GROUP III A

DEPLETED RATS INOCULATED INTRAPERITONEALLY WITH AVIAN TUBERCLE BACILLI

(Dose 1 mgm.)

Rat No.	Duration of Life after Inoculation	Authopsy Findings
1	33 days	No tuberculosis.
2	30 days	No tuberculosis.
3	20 days	No tuberculosis.
4	20 days	No tuberculosis.
5	18 days	Tuberculosis of peritoneum and mesentery with advanced caseation.
6	19 days	Tuberculosis of peritoneum and mesentery with caseation.
7	10 days	Tuberculosis of peritoneum and mesentery.
8	14 days	Tuberculosis of peritoneum and mes entery with early caseation.

Average duration of life after inoculation—19.2 days.
Percentage of infection—50%

TABLE II

GROUP III B

PARTIALLY DEPLETED RATS INOCULATED INTRAPERITONEALLY WITH AVIAN TUBERCULE BACILLI

(Dose 1 mgm.)

Rat No.	Duration of Life after Inoculation	Autopsy Findings
1	56 days	No tuberculosis
2	54 days	No tuberculosis
3	34 days	No tuberculosis
4	54 days	No tuberculosis
5	54 days	Tuberculosis of lymph gland at hilus of spleen.
6	54 days	Tuberculosis of peritoneum, omentum and mesentery.
7	56 days	Tuberculosis of peritoneum.
8	56 days	Tuberculosis of peritoneum.

Rats killed 54 to 56 days after inoculation; one died on 34th day.
Percentage of infection—50%

TABLE III

GROUP III C

RATS ON ADEQUATE DIET INOCULATED INTRAPERITONEALLY WITH AVIAN TUBERCLE BACILLI
(Dose 1 mgm.)

Rat No.	Autopsy Findings
1	Tuberculosis of mesenteric lymph gland.
2	Tuberculosis of portal lymph gland.
3	No tuberculosis
4	No tuberculosis
5	No tuberculosis
6	No tuberculosis
7	Tuberculosis of peritoneum.
8	Tuberculosis of peritoneum.

All rats killed 49 days after inoculation.
Percentage of infection—50%