

# THE IMMUNOLOGICAL EFFECTS OF COCONUT FACTOR IN EXPERIMENTAL TUBERCULOSIS OF MICE

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## ABSTRACT

A polysaccharide factor isolated from coconut water, when injected into mice, induces a moderate but statistically significant degree of resistance to experimental tuberculosis. The factor also gives an antigen-antibody reaction with serum from tuberculosis infected guinea pigs showing that it is probably immunogenic.

It has been known that killed tubercle bacilli are immunogenic in experimental animals, to tuberculosis.<sup>1-4</sup> This immunity differs from that against other viral and bacterial diseases like small-pox, etc., in that the protection derived from vaccination against tuberculosis can be more easily overcome. However, one of the fundamental concepts in immunology is that immunity against any disease can be defeated by severe enough infection<sup>5, 6</sup> and when this happens, whether or not immunity is present can be measured only by comparing the severity of the disease in vaccinated and nonvaccinated animals.<sup>7</sup> This latter method has been widely used in immunological experiments in tuberculosis.

Though the whole cells of killed tubercle bacilli have thus been shown to be immunogenic, the actual component in the cell which is responsible for the immunity has not been isolated. Toda and Murata<sup>8</sup> have reported that injections of tuberculin polysaccharide can induce a moderate but definite degree of resistance to tuberculosis though this has not been confirmed by other workers.

Ramakrishnan *et al.* reported<sup>9, 10</sup> that a polysaccharide factor isolated from coconut water has the property of promoting the growth of *M. tuberculosis in vitro*, but when injected into mice infected with the tubercle bacilli, actually prolongs the survival period. One of the plausible reasons for this conflicting property is that the coconut factor contains an immunogenic antigen which is also present in the tubercle bacilli and, when injected into infected animals, produces certain degree of immunity against tuberculosis. Experiments have been carried out to test this possibility. These preliminary results, presented in this paper indicate that a moderate but statistically significant degree of resistance against tuberculosis can be induced by injections of the polysaccharide factor from coconut water and that this factor gives an antigen-antibody reaction with serum from experimental animals infected with virulent tubercle bacilli of human type. As far as the authors are aware, this is the first reported account of polysaccharide from a plant source exhibiting prophylactic activity against tuberculosis.

#### MATERIALS AND METHODS

The coconut factor was prepared by the method already described.<sup>9, 10</sup> The material was dissolved in water such that 0.2 ml. of the solution contained 15 mg. of solid. This solution was diluted further when required.

*Antibody serum*—Tuberculin negative, healthy guinea pigs weighing 350 to 500 gms. were selected and infected intramuscularly with 0.1 mg. wet weight of 10 days old culture of *M. tuberculosis* grown in Youmans media. The progress of the disease was followed by noting the weights of the animals and tuberculin testing with O.T., 1/10, at intervals. When progressive disease was established as indicated by development of tuberculin hypersensitivity and loss in weight, blood was collected by heart puncture and the serum separated out. This constituted the antibody serum. Serum from uninfected guinea pigs similarly obtained was used as controls.

For *in vitro* experiments a modified agar precipitation technique of Oakley and Fulthorpe<sup>11</sup> was used. A sterile 1.6% agar gel in distilled water was melted and carbolized at 0.5%. After cooling to 50°C, three volumes of this were mixed with seven volumes of the carbolised antiserum (0.35 ml. undiluted antiserum with 0.15 ml. of agar gel) heated to 50°C. The mixture was delivered into test tubes (100 × 8 mm.) which had been warmed to the same temperature. They were placed in the cold till the bottom of the gel had set. 0.8% agar gel in distilled water (carbolized at 0.5%) was melted and afterwards cooled to 50°C. 1 ml. of this was poured over the serum agar column. After the central agar column had set, 0.5 ml. of progressive dilutions of a carbolized antigen was poured into the tube to allow the antigen to diffuse into the central agar column and to come in contact with the antibodies diffusing from below. The tubes were sealed with paraffin-soaked cotton wool stoppers and kept at room temperature. The reactions were read after 24 hours, 48 hours, etc.

*In vivo experiments*—Albino mice whose susceptibility to tuberculosis with human strain of *M. tuberculosis* had been established earlier were the experimental animals.

Adult mice weighing 20 to 30 gms. were divided into two groups (A and B) of twenty each. They were housed in individual cages and maintained on laboratory diet given *ad lib*.

For studying the immunological effect of the polysaccharide fraction, three doses of this factor, 5 mg., 10 mg., and 15 mg., respectively, dissolved in 0.2 ml. of water were given intraperitoneally to the mice in group A. The second group received a similar dose of saline. A period of one month was allowed to lapse for the development of antibodies.

At the end of this period, each group was further subdivided into two:  $A_1$  and  $A_2$ ;  $B_1$  and  $B_2$ , each consisting of 10 animals. Groups  $A_1$  and  $B_1$  were infected intravenously with 0.1 mg. of *M. tuberculosis* ( $H_{37}R_1$ ) as described earlier.<sup>12</sup> Groups,  $A_2$  and  $B_2$  served as uninfected controls. The weights of

the animals were recorded at frequent intervals till the death of all infected animals. Macroscopic and microscopic examination of the organs was done to confirm the death as being due to only tuberculosis.

*In vitro antigen-antibody reaction*—The reaction was carried out by the method described under "Materials and Methods". The antiserum for the experiment was drawn from a guinea pig which was given intramuscular injection of live tubercle bacilli (0.1 mg, wet weight) 6—8 weeks earlier. For control, the antiserum was drawn from a normal healthy guinea pig. The antigen in both cases consisted of 15 mg. of coconut factor dissolved in 1.0 ml. of 0.1 N hydrochloric acid. After 24 hours, a ring of precipitate was formed in both test tubes and after another period of 24 hours a precipitate was formed in the test tube containing infected serum (Plate I). A corresponding precipitate was not formed in the control even after a week. From this it may reasonably be concluded that an antigen-antibody reaction takes place between the coconut factor and an antibody formed during the course of infection of tuberculosis in the experimental animal.

*In vivo experiments*—One of the normal criteria for assessing the progress of infection is the rapidity with which the infected animals lose weight and the weight curves generally indicate the enhancing or retarding effects of the immunogenic or chemotherapeutic agents. As could be seen in Fig. I, the injections

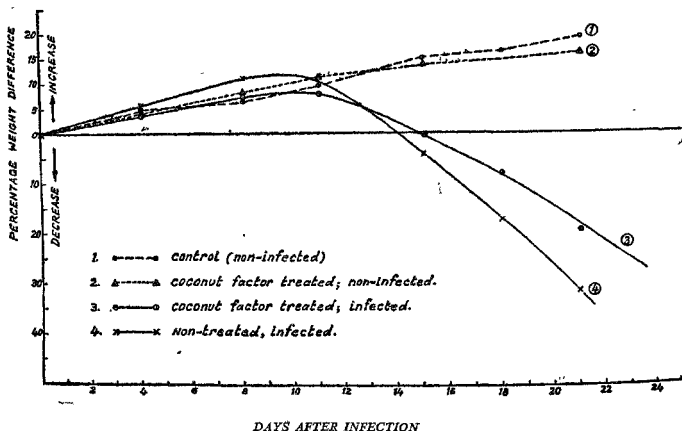


FIG. I

Influence of coconut factor on experimental tuberculosis of mice  
(Gain or loss in weight during the course of the disease)

of the polysaccharide factor have definitely reduced the weight loss and thus could be considered as exhibiting a restraining influence on the disease.

Prolongation of the survival period is another feature of prophylactic immunity. A statistically significant increase in the prolongation of life could be seen in the polysaccharide factor injected animals. (Table I). This observation

TABLE I  
The effect of immunity induced by coconut factor on tuberculosis of mice

Group	Number of animals	Mortality and survival time		ST <sub>50</sub> (Median survival Time) ± S.E.	t	p
		Number of deaths	Days after infection			
I (control infected).	10	10	21, 21, 21, 22, 22, 22, 22, 23, 23, 23.	21.5 ± 0.31		
II Experimental (Coconut factor immunised and infected).	10	10	23, 23, 25, 25, 25, 25, 25, 25, 26, 26.	24.0 ± 0.42	4.79	P < 0.01

Infection: Myco. tuberculosis (H<sub>37</sub>R<sub>v</sub>)  
dose: 0.1 mg. Route: I.V.

S.E. = Standard error  
S.T.<sub>50</sub> = Median Survival Time (ST 50)  
calculated by plotting the probits of % mortality against log days.

coupled with the weight curve point to the conclusion that the factor does induce a certain degree of resistance to tuberculosis.

#### DISCUSSION

The prevalent view, so far, on immunity in tuberculosis has generally been that the known antibodies which react either with the bacilli or products derived from them are unable to enhance the resistance of the animal. But recent studies have shown that humoral antibodies which may afford protection are present in the serum of infected animals though the nature of the antigens involved is not yet clear.<sup>13</sup>

Knowledge of the nature of the immunizing substance in tuberculosis would not only be of great importance in prophylactic immunization but would be of equal importance for the light it would shed upon the mechanism of acquired resistance to tuberculosis. From the experiments described above it can be concluded that a polysaccharide which is completely free of proteins and lipids can induce a significant resistance to tuberculosis. That substances other than pro-

teins could be antigenic and initiate production of antibodies has been demonstrated by other workers.<sup>14, 15</sup>

Unlike the chemoprophylaxis obtained by drugs like INH, which afford protection due to their antituberculous property, the nature of the resistant state induced by the coconut factor seems to be entirely different. It has previously been shown that this factor has no *in vitro* inhibitory action on tubercle bacilli and in fact facilitates the quicker growth of the mycobacteria on synthetic media. But, *in vivo*, the same factor prolongs the survival time to a slight extent, when administered during the course of the infection. A similar retarding effect is also now shown to be produced by prophylactic inoculation. Hence, its mode of action appears to be immunogenic rather than therapeutic.

The significant feature of these results is the production in mice of an immune state to tuberculosis by a polysaccharide factor from a plant source, which, in normal conditions would have been produced only by injections of the attenuated or killed micro-organisms.

These observations are essentially preliminary in nature. The extremely heavy doses of infection, used in our experiments, resulting in 100% mortality of the animals in a very short period is rarely met with in clinical conditions. Further experimentation on the course of less severe infections after prophylactic inoculations by the coconut factor would throw light on the immunogenicity of this factor and its therapeutic usefulness. Investigations on these aspects and the conditions under which this immunogenicity is maintained maximally for the longest period of time are being carried out.

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*Immunological Effects of Coconut Factor in Experimental Tuberculosis*



PLATE I

Antigen-Antibody reaction

- I Normal serum of guinea pig and polysaccharide factor (No precipitate seen)
  - II Serum from infected ( $H_{27}R_0$ ) guinea pig and polysaccharide factor
- Ring of precipitate observed (P)  
B. Antigen-agar gel interface  
P. Antigen-antibody precipitate

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