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# REEVALUATION OF
# RESEARCH NEEDS IN TUBERCULOSIS

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**PAN AMERICAN HEALTH ORGANIZATION**
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REEVALUATICN OF RESEARCH NEEDS IN

TUBERCULOSIS

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# THE PREPARATION AND STANDARDIZATION OF

## ANTITUBERCULOUS VACCINES'

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## I

Several different criteria have been used to evaluate the protective

efficacy of antituberculous vaccines. The most common are:

a) effect on survival time following exposure to virulent

infection;

b) histopathological characters of the disease;

c) fate of the virulent bacilli in vivo: their survival, their

multiplication, and their spread in the various organs.

It has been demonstrated beyond doubt that, irrespective of the

criterium used, the resistance of animals and of man to tuberculosis can be

greatly enhanced by prior administration of either:

a) living BCG bacilli;

b) preparations derived from killed bacilli.

## II

Although the possibility of increasing resistance to tuberculosis

by vaccination has thus been convincingly demonstrated, it is unfortunately

true that antituberculous vaccines often give disappointing results, both

in animals and in human beings. To a very large extent these failures arise

from the fact that many of the vaccines used are of low protective activity,

or even are entirely inactive.

It is a surprising, and very regrettable fact, that no national or
international organization has formulated meaningful requirements of activity
for antituberculous vaccines. For this reason it seems justified to briefly
outline some of the factors which are known to condition the protective
activity of vaccines.

### III

Although the dose of BCG vaccine is always defined in terms of weight
or volume units, these units have no useful meaning because the immunogenicity
of the material injected is determined not by the quantity, but rather by
qualitative characteristics of the vaccine, in particular:

a) number of viable bacilli per unit weight or volume;

b) age, and physiological activity, of the living bacilli;

c) genetic properties of the BCG culture used for the preparation
   of the vaccine. With regard to this last point, it must be
   emphasized that the original BCG culture has undergone, and
   continues to undergo, mutational changes, which give rise to
   a multiplicity of substrains differing greatly in behavior, both
   in vitro and in vivo.

In addition to the difficulties arising from the differences in
immunogenicity corresponding to the factors just mentioned, other difficulties
come from the fact that living BCG vaccines rapidly lose their activity, unless
stabilized by desiccation. Naturally, it is possible to obtain BCG vaccine
in a dried active form by lyophilization. But this technique presents technical
difficulties, and for this reason is not as widely used as would be desirable.
Yet it is almost certain that standardization of BCG vaccine will not become
a useful reality until it is carried out on stable, desiccated preparations.

IV

When properly carried out, prior administration of vaccines made from
killed tubercle bacilli, or from extracts thereof, can increase the survival
time of experimental animals infected with virulent bacilli. However, the
same result can be obtained by administration of other unrelated biological
products - for example, of endotoxins derived from Gram negative bacilli.
For reasons that cannot be discussed here, increase in survival time is not
an adequate criterium of immunity.

In order to produce true antituberculous immunity a killed vaccine
(just as well as a living BCG vaccine) must elicit a kind of response which
interferes with the spread and the multiplication of virulent bacilli in the
vaccinated animal. There is highly suggestive evidence that this kind of
response has been elicited with a few preparations of killed vaccines, but
studies on this score are still in a very primitive state. Moreover, no
knowledge whatever is available concerning the nature of the bacterial
constituents which elicit true immunity.

CONCLUSIONS

The problems posed by the preparation and use of antituberculous
vaccines are of a very different nature depending upon the type of vaccine
under consideration.

In the case of the living BCG vaccine most of the fundamental work
has been done. The problems to be solved are of a developmental rather than
of a theoretical nature. They involve:

    a) selection and maintenance of a BCG substrain having the proper
        degree of activity;

b) definition of the optimum physiological state (age in particular)

   of the bacilli;

c) number of viable bacilli to be used per dose;

d) development of reliable methods of lyophilization;

e) the solution of these practical problems will lead automatically

   to the standardization of the vaccine.

The situation is very different with regard to killed vaccines. In this case it will be necessary to first carry out theoretical studies in order to determine:

a) how bacilli can be killed without destroying their protective

   efficacy;

b) the nature of the bacterial constituents which elicit true

   immunity (as against non-specific alteration of response to

   infection).

Both in the case of living BCG and of killed vaccines, there is also need for theoretical studies concerning:

a) route, time, and frequency of vaccination;

b) measurement of the immune state in the vaccinated individual.

While it would be fairly easy to formulate a plan of experimental studies on vaccination, it will probably prove more difficult to implement such a plan in practice. Difficulties will come from the fact that, contrary to general belief, very few institutions have proper facilities for the handling of virulent tubercle bacilli, and for the maintenance of infected animals during prolonged periods of time. Even more important is the fact that the study of tuberculosis is not a popular subject, especially in the

U.S.; few are the well trained scientists who are willing to dedicate
themselves to this problem. It would seem that a program of international
fellowships, with long term tenure and support for experimental work, will
be necessary before one can solve the theoretical and practical problems
involved in antituberculous vaccination.

# NUTRITION AND TUBERCULOSIS

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A relation between nutrition and tuberculosis has been postulated since the earliest studies on the disease. This was based on clinical and epidemiological evidence. It has been clearly established by many observations that tuberculosis mortality rates increase during war and famine. The data from World War I and II have been summarized by Keys et al[1].

The most effective treatment for the disease before chemotherapeutic agents were developed was rest, fresh air, sunshine and good food.

However, these empirical observations fail to establish any real connection. In times of war and famine many other changes occur simultaneously. There is failure to isolate open cases, overcrowding, lack of adequate housing and clothing, and other factors which may influence mortality from the disease.

In evaluating treatment, good nutrition was regarded as synonymous with an increase in body weight and the diet in many institutions was, for many reasons, a high carbohydrate diet rather than a high protein one. The varying reaction of the individual to the infection made clinical observations difficult to interpret and the concept of host resistance was developed without clarification of the factors on which host resistance depended. Loss of weight could be explained on the basis of lack of appetite, fever and toxicity.

Many experiments were carried out on experimental animals of various kinds and with various specific deficiencies and there is an extensive literature on the subject. Reference will be made only to a few selected studies.

Numerous experiments have been done on the effect of Vitamin C deficiency on tuberculosis in the guinea pig. One of these by McConkey and Smith [2] showed less infection when the animals received enough ascorbic acid. Other studies have also shown an apparent favorable effect from adequate Vitamin C. Greene [3] et al. studied the role of chronic Vitamin C deficiency in tuberculosis in the guinea pig and found that a shortened survival time and more rapid development of generalized tuberculosis in the animals with chronic Vitamin C deficiency.

Numerous experiments with Vitamin A deficient diets were also carried out. One of these by Finkelstein [4] showed that mortality was highest and occured earlier in mice infected with tuberculosis.

Skiramachari and Gopolan [5] also observed in rats on a Vitamin A deficient diet that incidence of infections were higher and the lesions more extensive, although restriction of calorie intake by 50% did not significantly influence mortality or resistance to infection. Protein deficiency also did not influence the resistance of guinea pigs or rats to infection or mortality within 7 to 12 weeks although animals on high protein showed earlier recovery of nitrogen balance.

Studies with Vitamin D deficiency in rabbits by Steiner et al. [6] showed that rachitic rabbits did not have any more extensive lesions and the survival time was not shortened for the controls.

Merrick and Ratcliffe [7] did a careful study on the effect of the level of dietary protein in isocaloric diets fed to hamsters who were

infected by inhalation of small nuclei of tubercle bacilli of human

origin. They used levels of approximately 30%, 17% and 6% protein, although

the variation in protein level did not affect susceptibility nor modify the

progress of the disease by organisms of high virulence until about 130 days

after infection, Beyond 130 days, progress of tuberculosis was most rapid in

the animals on the 6% protein diet whereas on 30% protein the disease was

arrested or regressed for a time. They concluded that the level of dietary

protein can be a critical factor in the development of resistance to

tuberculosis but that prolonged interaction of host and organism is necessary.

The failure to obtain any difference in the first 130 days is interpreted

as evidence against native or hereditary resistance.

The series of papers from Dubos and his associates have focused

attention on host resistance and, more particularly, on nitrogen and protein.

Attention also has been given to calcium and dietary lipids in

experimental animals. Hedgecock [8] has reported that mice fed a lipid-free

diet or one containing 20% of coconut oil were retarded in the progress of

tuberculosis while those fed 20% olive oil, linseed oil and oleic acid had

an enhancement of the progress of the experimental tuberculosis. This

suggests an effect from the quantity of unsaturated fatty acids in the

diet.

From the standpoint of the role of nutrition in tuberculosis in

experimental animals it may be said that, in spite of the hundreds of

papers that have been written on the subject, we still do not know the

exact relationships. The infection in animals differs from that in man.

The nutritive requirements of different species of animals differ widely

as does their susceptibility to tuberculosis infection. More precise and

carefully controlled studies still need to be done.

Because of the difficulties in interpreting animal data, more specific reference will be made to human studies.

As a result of the experimental work on animals many cases of human tuberculosis were given cod liver oil and tomato juice in order to increase the plasma Vitamin A and C levels. Although unusually large doses of these vitamins are required for saturation in tuberculosis, the relationship between them and the infection remains obscure. For example, Pijoan and Sedlacek[9] have shown that in Navaho Indians with tuberculosis there was a greater than normal demand for Vitamin C.

Getz and Koerner[10] in 1941 found low plasma levels of Vitamin C and Vitamin A in tuberculous patients and concluded that tuberculosis makes heavy demands on the body for these vitamins in proportion to the extent of the disease.

Crimm and Short[11] found at autopsy on 50 cases of tuberculosis that the liver approached depletion of Vitamin A in 14% of the cases, and Breese, Watkins and McCoord[12] found some impairment of absorption of Vitamin A in tuberculosis.

Getz, Long and Henderson[13] did a very careful study of a group of 1,100 men for a period of time up to five years during which 28 developed X-ray evidence of tuberculosis. Numerous blood studies were done including calcium, phosphorus, protein and hemoglobin. All of the values were evenly distributed throughout the group except for Vitamins A and C. There was an inverse relationship between the development of tuberculosis and the blood levels of these vitamins.

Brewer et al.[14] carefully studied riboflavin, thiamine and nitrogen metabolism in 6 women with active tuberculosis and found it to be similar to the metabolism in women free of tuberculosis. They think

that the dietary problem in tuberculosis may be primarily one of failure

of the patient to consume an adequate amount of nutrients. A later report[15]

of further studies on these same cases showed that they had an increased

requirement for calcium.

One of the most interesting areas of relationship between

nutrition and tuberculosis is that of protein and nitrogen balance and

several studies have been done in man.   Johnston [16,17] has reported

nitrogen balance studies in adolescents with tuberculosis and has observed

that the spread of the disease is associated with a failure of nitrogen

retention and that the course of the disease correlates with nitrogen

storage.

Co Tui, Kuo and Schmidt[18] also studied nitrogen balance and

rapid weight gain. Four of the patients were in significantly positive

balance at the start. There is no evidence that the tuberculosis patient

requires a higher than normal nitrogen intake to attain nitrogen

equilibrium.

Nitrogen balance studies are difficult to interpret because of

the many factors which affect them, such as bed rest and emotional upsets.

Gilliland et al.[19] have studied the serum proteins in tuberculosis

by electrophoresis, using 327 adults with pulmonary tuberculosis. The

study shows  that there is a decrease in serum albumin with a rise in alpha-

2-globulin so that the albumin/alpha-2-globulin ratio reflects the extent

and activity of the infection and the recovery of the patient.

The use of isoniazid in relation to nutrition is particularly

interesting for two main reasons. The drug may produce toxic symptoms

which can be alleviated by the administration of pyridoxine. Therefore,

at least one of the actions of the drug is that of a metabolic inhibitor

of a normal metabolic pathway. Whether the therapeutic effect of isoniazid may be from the accumulation of an abnormal metabolite is an interesting speculation. Also, the drug stimulates appetite leading the patient to greater food consumption, increased weight gain and presumably better nutrition which in itself may be having a therapeutic benefit.

Therefore, in the area of nutrition and tuberculosis we have little exact knowledge either as to specific nutritive relationships with the growth of the organism, its toxin production, the resistance of the host, the development of antibodies, the effectiveness or reaction to vaccination or the combined effect with chemotherapeutic agents. There are vast fertile areas for research involving prevention as well as therapy which should be undertaken especially in connection with programs of prevention as well as therapeutic regimes.

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