THE PRESENT STATUS OF TECHNICAL KNOWLEDGE CONCERNING IMMUNIZATION AGAINST TUBERCULOSIS[1]

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Long and involved controversy surrounds the subject of BCG vaccination. This article provides a thorough review of the issues involved, and describes major factors influencing the effectiveness of BCG vaccine.

Introduction

For about half a century immunization against tuberculosis has been subjected to comprehensive scientific investigation, and during the last three decades about 300 million children have been vaccinated against the disease. An enormous amount of knowledge and practical experience has thus been accumulated, and yet immunization against tuberculosis is a matter of controversy. Widely differing viewpoints still leave room for disputes which occasionally evoke strong emotions.

The story of BCG vaccination is full of dramatic conflicts and controversies. This may partly explain why on the one hand there are countries where BCG vaccination has been adopted as legally compulsory national policy, providing coverage for nearly 100 per cent of the eligible population, while on the other there are a few countries where BCG vaccination has been practically unknown to the public and has been unpopular or almost taboo within the medical profession. That, after 50 years of research, is the rather confusing present state of affairs.

This presentation will try to give a balanced account of the facts and attempt their interpretation. The goal is not to describe completely, but to focus on those findings which could provide a scientific basis for framing national policies, i.e., on technical information which appears relevant for the public health decision-maker.

Immunological Background Information

The Rationale for Vaccination Against Tuberculosis

At the beginning of this century, it was often noted that almost everybody got infected with tubercle bacilli, but that only a relatively small proportion of people developed clinically manifest tuberculosis. The large majority of those infected, who did not develop the disease, seemed to have acquired a certain resistance to tuberculosis, although subsequent infections with virulent tubercle bacilli were unavoidable and frequent at the time.

This observation, confirmed by experiments in animals and the application of immunological principles pertaining to other infectious diseases, led to the following hypotheses:

a) Primoinfection is capable of preventing subsequent infections from developing into clinical disease.

b) Artificial primoinfection could have the same effect. However, since virulent tubercle bacilli are potentially harmful, a strain of mycobacteria should be substituted which is not pathogenic for man.

These were Calmette's principal reasons for selecting a strain of *Mycobaterium bovis*, which after many years of serial culturing (231 passages) had lost all its pathogenicity for man. Calmette and Guerin, the two men who isolated it, considered it a *"virus fixé."* Since then, numerous attempts have been made to find out whether its original pathogenic capacity might

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BCG vaccination of a Costa Rican schoolboy (WHO photo).

be restored. Fortunately, these efforts have been in vain, and the organism has remained an attenuated bacillus. Just half a century ago it was tried out in man for the first time. Today all existing BCG vaccines are derived from this attenuated strain.

At the same time, however, it is important to note an entirely contrary hypothesis that has been widely promoted, especially in this Hemisphere.

The main tenet of this hypothesis is that reinfection of persons who are already sensitized to tuberculoprotein leads to the progressive adult type of tuberculosis. As stated by one author:

> Tubercle bacilli of reinfection are likely to result in clinical disease because their invasion occurs on allergic tissues. Before allergy appears, tuberculoprotein is innocuous to cells and tissues. With the appearance of allergy, however, it becomes a violent poison and thus may kill cells and tissues with which it comes in contact.
>
> Obviously, therefore, both in animals and humans the defense mechanism operates more effectively against initial invasions than those which later occur in allergic tissue. To produce allergy artificially, even with dead tubercle bacilli, can be a dangerous procedure for many persons. (Myers, 1957.)

This unquantified hypothesis, however, seems not to have withstood the test of time. BCG vaccination has not been proved harmful and in fact is considered to be one of the safest vaccinations in man. Recent works state that sensitization through widespread infection with nonspecific mycobacteria is not dangerous for man but can, on the contrary, have a beneficial effect like a "natural vaccination" against tu-

berculosis (Palmer and Long, 1966; Palmer and Edwards, 1968). Palmer and his group thus express clear disagreement with Myers' hypothesis by interpreting nonspecific sensitivity as a sign of acquired resistance rather than a sign of danger.

Some Immunological Findings

The immune response in tuberculosis, particularly as it relates to delayed hypersensitivity, has long been a controversial subject. Numerous attempts have failed to produce a vaccine[3] from live, killed, or fractionated mycobacteria which confers protection without inducing delayed hypersensitivity. However, there is growing evidence to support the hypothesis of Dubos and Pierce (1956) that only live, metabolically active mycobacteria multiplying in the host can produce immunity of the required strength and duration. For many years it has been known that delayed hypersensitivity cannot be passively transferred by serum, but only by lymphocytes from sensitized individuals; however, in recent years even acquired resistance has been transferred in that way. From this and other evidence it is possible to conclude (Mackaness, 1968) that both hypersensitivity and acquired resistance are not only cell-mediated but also far more closely related than was once commonly believed.

The discovery of non-tuberculous mycobacteria with immunizing capacity has added another immunologic consideration to the subject. It is now generally accepted that low-grade tuberculin sensitivity is mainly due to sensitization by mycobacteria other than *M. tuberculosis* (Palmer and Strange Petersen, 1950; Edwards *et al.*, 1955; WHO Tuberculosis Research Office, 1955a, 1955c).

Such low-grade or nonspecific tuberculin sensitivity is particularly prevalent in tropical areas, more frequent in lowlands than in highlands, and more frequent in men than in women (Nyboe, 1966). The prevalence of this sensitivity also increases with age.

Studies of delayed hypersensitivity to purified protein derivatives (PPDs) prepared from mycobacteria of Runyon Groups I-IV indicate that the causative agent of low-grade sensitivity in man may be antigenically close to mycobacteria of Runyon Groups II and III, e.g., *M. gause, M. avium,* or *M. battey* (WHO Tuberculosis Research Office, 1955b; Edwards *et al.*, 1962; Hart *et al.*, 1962; and Edwards *et al.*, 1965).

In a study on mice (Youmans *et al.*, 1961) and a large experiment on guinea pigs (Palmer and Long, 1966) a BCG-like effect of varying degrees was caused by certain mycobacteria of Runyon Groups I-IV. Interestingly, the degree of protection was shown to be closely related to the average strength of sensitivity to PPD-S[4] induced by the respective mycobacteria. However, the protection conferred by BCG was always found to be stronger than that conferred by the atypical mycobacteria. When BCG was administered to guinea pigs already immunized by nonspecific mycobacteria, the protective effect was stronger but not additive. It was simply as strong as if BCG had been given alone—no stronger, no weaker.

Differentiating Mycobacterial Infections

It is obviously of great epidemiologic interest to distinguish between members of a population infected by *M. tuberculosis,* those infected by nonspecific mycobacteria, and those remaining uninfected. Where only one kind of tuberculin is being employed, individuals who react only to a strong dose such as 100 tuberculin units of PPD-S, and not to a weak dose such as 5 tuberculin units, could be classified as having low-grade sensitivity; this means they are probably infected by nontuberculous mycobacteria. Nowadays, simultaneous testing with two or more antigens is preferred; these should be PPDs prepared from

[3]Individual laboratories have reported producing such vaccine, but other laboratories have been unable to confirm or reproduce their results.

[4]Purified Protein Derivative—Standard.

atypical mycobacteria, including one mammalian PPD. The most extensive experience until now has been with PPD-B (Battey) prepared from a non-chromogenic mycobacterium of Runyon Group III, applied in a dose of the same biological strength as that of its companion, PPD-tuberculin. Although testing with multiple antigens provides us with more information than testing with PPD-tuberculin of various strengths, it lacks the discriminative capacity needed for individual diagnosis.

The Committee on Diagnostic Skin Testing, in its statement on the tuberculin test (Medical Section of the National Tuberculosis and Respiratory Disease Association, 1971), stressed that "simultaneous skin testing with PPD tuberculin and an antigen prepared from an atypical mycobacterium is of *some help* [author's emphasis] in making a differential diagnosis between reactivity caused by infection with *M. tuberculosis* or other mycobacteria." Because of the low specificity of the antigens, however, it is practically impossible to determine which *particular* mycobacterium is causing low-grade skin sensitivity in cross-reaction with PPD-tuberculin.

It should also be noted that it has not yet been possible to differentiate BCG-induced sensitivity from postinfectious sensitivity caused by *M. tuberculosis*. BCG, which was originally a strain of *M. bovis*, appears antigenically related to *M. tuberculosis*. Multiple testing with PPDs prepared from atypical nontuberculous mycobacteria has not helped to distinguish between these two close mammalian types of mycobacterial infection (Comstock *et al.*, 1970).

Administration of PPDs prepared from nontuberculous mycobacteria, and simultaneous administration of PPDs from tubercle bacilli, are helpful primarily in group diagnosis. For individual diagnosis, very great caution must be used in interpreting test results.

The Efficacy of BCG Vaccination in Man

Vaccination Trials

The most valuable contribution to our knowledge on vaccination efficacy has come from controlled trials. In those reported here, individuals eligible for vaccination were divided at random into two groups; one group received the vaccination while the other received a placebo and served as the control group. Standard follow-up procedures were established, and those making observations had no knowledge of whether an individual belonged to the vaccinated or the placebo group. The results of seven such controlled trials are presented in Table 1.

The figures shown in the last column indicate striking variations in protective efficacy,[5] ranging from 0 to 80 per cent. A trial in Puerto Rico indicated that BCG vaccination had a moderate effect, but trials in Georgia (1947) and in Georgia and Alabama (1950) showed it to have very little or no effect. Another trial in Southern India showed 60 per cent efficacy after 7-1/2 years, declining to 31 per cent after 12-1/2 years. On the other hand, a trial in a population of North American Indians showed high protective efficacy on the order of 80 per cent. Similar high levels were attained by trials in Chicago infants and in British adolescents 14 to 15-1/2 years old. These conflicting results have naturally provoked intense discussion.

Effective measures were taken to prevent methodological shortcomings from affecting the allocation of eligible subjects or the assessment of results, and all the trials had built-in safeguards against bias. Furthermore, genotypical or phenotypical differences between the populations belonging to various ethnic groups and countries were found not to be responsible for the large differences in efficacy (Sutherland, 1967).

The suggestion that gross malnourishment or nutritional differences could have reduced the efficacy of vaccination, as had been the case in mouse experiments (Dubos, 1964), was not in accord with the high vaccination efficacy in

[5] Protective efficacy is the percentage difference between the annual incidence of disease in the unvaccinated and the vaccinated groups. It can also be expressed as the reduction of the annual incidence of disease in the vaccinated group as compared with the control group.

TABLE 1—Results of seven controlled trials of BCG vaccination against tuberculosis.

Population group and reference	Period of intake and age-range	Criteria establishing eligibility for vaccination	Source of vaccine	Duration of follow-up (years)	Vaccination group	No. of subjects	Cases of tuberculosis		Protective efficacy (per cent)
							No.	Rate[a]	
1) North American Indians (8 tribes) (Stein & Aronson, 1953)	1935-1938 0-20 years	A negative reaction to 0.005 mg of PPD-Seibert (250 TU)	Henry Phipps Institute, Philadelphia	9-11	Unvaccinated BCG	1,457 1,551	238 64	1,563 320	80[b]
2) Chicago infants in high-risk areas (Rosenthal *et al.*, 1961)	1937-1948 Under 3 months	No prior tuberculin testing	Tice Laboratory, Chicago[c]	12-23	Unvaccinated BCG	1,665 1,716	65 17	223[d] 57[d]	75
3) Georgia, general population (Comstock & Webster, 1969)	1947 6-17 years	A reaction of under 5 mm to 0.002 mg of RT 18 (100 TU)	Tice Laboratory, Chicago[c]	20	Unvaccinated BCG	2,341 2,498	3 5	11 17	None
4) Puerto Rico, general population (Palmer *et al.*, 1958)	1949-1951 1-18 years	A reaction of under 6 mm to 0.0002 mg of RT 19-20-21 (10 TU)	N.Y. State Department of Health, New York	5 1/2-7 1/2 (mean: 6.3)	Unvaccinated BCG	27,338 50,634	73 93	43 30	31
5) Georgia and Alabama, general population (Comstock & Palmer, 1966)	1950 5 years and over	A reaction of under 5 mm to 0.0001 mg of RT 19-20-21	Tice Laboratory, Chicago[c]	14	Unvaccinated BCG	17,854 16,913	32 26	13 11	14
6) Great Britain, urban population (BMC Report, 1972—see Tables 2 and 3)	1950-1952 14-15 1/2 years	A reaction of under 5 mm to 0.1 ml of 1% Old Tuberculin (100 TU)	Statens Serum-Institut, Copenhagen	15	Unvaccinated BCG	12,699 13,598	240 56	128 28	78
7) Southern India, rural population (Frimodt-Moller *et al.*, 1968)	1950-1955 All ages	A reaction of under 5 mm to 5 TU of RT 19-20-21	BCG Laboratory, Madras	9-14 (mean: 12.3)	Unvaccinated BCG	5,808 5,069	46 28	89 61	31-60[e]

[a]Annual rate per 100,000 population, usually allowing for observation losses.
[b]The protective efficacy against death from tuberculosis was 82% for a period of 18-20 years (Aronson *et al.*, 1958).
[c]This laboratory has issued a number of strains at different times, and it is not known whether the strains used in these three trials were the same or not.
[d]Assuming a mean observation period of 17.5 years.
[e]60 per cent efficacy after 7.5 years, 31 per cent after 12.5 years.

Source: Fourth Report of the British Medical Council on BCG and Vole Bacillus in the Prevention of Tuberculosis in Adolescence and Early Adult Life, 1972.

North American Indians (Hart and Sutherland, 1965). Moreover, the thickness of the subcutaneous fat layer was measured in the Georgia and Alabama trial, and no association with efficacy could be found (Comstock and Palmer, 1966).

It has also been suggested that the different results of the British trial and the trials in Georgia, Puerto Rico, and Georgia-Alabama can be explained by differences in the prevalence of nonspecific mycobacterial infection in the respective areas (Ferebee and Palmer, 1966; Palmer and Long, 1966). It can indeed be assumed that a portion of subjects in the American areas who had been classified as eligible had already been infected by nonspecific mycobacteria. Thus, they were likely to have acquired some immunity that could be increased only a little by BCG–a situation analogous to that in previously cited animal experiments (Youmans, 1961; Palmer and Long, 1966). Also, in the British trial a portion of the subjects were apparently already infected by nonspecific mycobacteria and thus had some naturally acquired immunity (see Tables 2 and 3).

The group of nonspecific reactors[6] in the British trial had about twice the disease incidence of those vaccinated with BCG. At the same time the control group, i.e., those with negative reactions who did not receive BCG, had about twice the disease incidence of the nonspecific reactors. These findings tend to agree with those of Palmer and Long (1966) showing that the population in the Georgia-Alabama trial was infected with mycobacteria having about half as much antituberculous potency as BCG. A similar degree of protection was observed in a follow-up study of Navy recruits (Edwards and Palmer, 1968).

However, if this estimate of BCG-like protection from nonspecific natural infection is correct, then the differences observed in the American study could not be explained as suggested. If we calculate the natural protection conferred by the nonspecific mycobacteria it would amount to about 95 per cent of that conferred by BCG (Hart, 1969).[7] This obviously conflicts with the initial hypothesis, since such a strong protective effect has never been found, either in experiments or in nature. Moreover, in the Georgia trial the effects of nonspecific mycobacterial infection can be discounted, because only nonreactors to 100 TU were eligible, as in the British trial.

If the differences under discussion cannot be explained by the frequency of nonspecific mycobacterial infection, then the only possible conclusion is that the vaccine used in the Georgia-Alabama trial was of lower potency than that used in the British trial. The batches of BCG supplied for the British trial were checked regularly, and their quality was generally found to be good, with a few exceptions. However, the potency of strains used to supply vaccine for the American trials was found to vary. Several studies in animals by two research laboratories revealed that cultures derived from one of the vaccines had very little ability to multiply, sensitize,[8] and protect (Suter and Dubos, 1951; Dubos, Pierce, and Schaefer, 1953; Dubos and Pierce, 1956; Willis *et al.*, 1960; Willis and Vandiviere, 1961; Jespersen, 1971). These several studies together indicate that the particular vaccine used in the American trials was prepared from a strain which did not multiply well in laboratory animals and did not protect them satisfactorily. It might be argued that many of the tests were carried out some years after the trials. However, when the strains used in the North American Indian trial and the Danish strain used in the British trial were tested many years later they appeared to have retained their previous virulence and their ability to sensitize and protect test animals. It can thus be concluded that the low BCG

[6]Subjects having skin reactions to 100 tuberculin units of Old Tuberculin, but not to 3 tuberculin units. (They were not vaccinated but were followed up.)

[7]It should be noted that in almost all tropical areas where nonspecific sensitivity is widespread the tuberculosis morbidity and mortality is high as well. If nonspecific sensitivity is synonymous with protection then the degree of protection must be low and insufficient.

[8]The postvaccination tuberculin sensitivity in man was also rather low.

TABLE 2–Cases of tuberculosis starting within a trial's 15-year follow-up period.

Section[a]	Tuberculin sensitivity of trial groups and vaccinations administered	Number of participants	Cases of tuberculosis: Number starting within 15 years	Cases of tuberculosis: Annual incidence per 1,000 participants[b]	Protective efficacy (%)
1) Children given BCG vaccine and those admitted concurrently with them	Negative, unvaccinated	12,699	240	1.28	
	Negative, vaccinated with BCG	13,598	56	0.28	78.4
	Positive to 3 TU of Old Tuberculin	15,514	204	0.89	
	Positive only to 100 TU of Old Tuberculin	6,153	52	0.57	
2) Children given vole bacillus vaccine and those admitted concurrently with them	Negative, unvaccinated	5,889	130	1.50	
	Negative, vaccinated with vole bacillus	5,817	25	0.29	80.8
	Positive to 3 TU of Old Tuberculin	8,783	118	0.91	
	Positive only to 100 TU of Old Tuberculin	3,068	32	0.70	
3) Children admitted concurrently and given either BCG or vole bacillus vaccine	Negative, vaccinated with BCG	5,581	17	0.20	
	Negative, vaccinated with vole bacillus	5,497	21	0.26	

[a]Many participants and cases of tuberculosis appear in more than one of the three separate sections of this table; therefore the figures from the different sections cannot be totalled.

[b]After allowing for reduction of the population at risk; i.e., through death or contraction of tuberculosis.

Source: Fourth Report of the British Medical Council on BCG and Vole Bacillus in the Prevention of Tuberculosis in Adolescence and Early Adult Life, 1972.

TABLE 3–Cases of tuberculosis starting within 15 years, classified according to the form of the disease.

Trial group	Total cases	Form of tuberculosis											
		Pulmonary tuberculosis, non-miliary		Tuberculous pleural effusion[a]	Hilar gland enlargement[b]	Tuberculous meningitis	Pulmonary tuberculosis, miliary	Bone or joint tuberculosis	Tuberculous adenitis	Tuberculous peritonitis	Erythema nodosum	Genito-urinary tuberculosis	Other forms[c]
		No.	%										
Negative, unvaccinated	243	163	67	51	2	5[d]	5[d]	3	4	2	4	3	1
Negative, vaccinated with BCG	56	40	71	8	1	0	0	2	1	1	1	2	0
Negative, vaccinated with vole bacillus	25	20	80	4	0	0	0	1	0	0	0	0	0
Positive to 3 TU of Old Tuberculin	206	143	69	14	0	1	1	6	22	1	0	16	2
Positive only to 100 TU of Old Tuberculin	53	40	75	8	0	0	0	2	1	1	0	1	0
Total (all groups)	583	406	70	85	3	6	6	14	28	5	5	22	3

[a] Without evidence of pulmonary tuberculosis.

[b] Without other evidence of tuberculosis.

[c] One case each of tuberculous bronchiectasis, tuberculous endobronchitis, and lupus vulgaris.

[d] In all, there were 10 cases of tuberculous meningitis and pulmonary miliary tuberculosis among the unvaccinated participants and none among vaccinated ones.

Source: Fourth Report of the British Medical Research Council on BCG and Vole Bacillus in the Prevention of Tuberculosis in Adolescence and Early Adult Life, 1972.

efficacy in the Georgia and Georgia-Alabama trials could have been due primarily to the properties of the vaccine used; it is also conceivable that underdosage may have played a significant role in the trials, which employed the multiple puncture vaccination technique.

The findings of these long-term trials are of fundamental significance for several reasons: they have provided practical evidence of the protective efficacy of BCG vaccination in man; they have supported the evidence that nonspecific sensitivity is associated with a certain degree of protection against tuberculosis; and they have focused attention on the overwhelming impact that variations in vaccine quality can have on efficacy.

Retrospective Studies

Numerous retrospective studies on BCG vaccination have been published; from among them, it may be useful to mention a few from countries where statistics on tuberculosis morbidity have been recorded for many decades.

One comparative study (Bjartveit and Waaler, 1965) analyzed morbidity rates in three Scandinavian countries where BCG vaccination had been widely used, but where national vaccination policies had differed. Since the 1940's, primary vaccination had been given systematically to newborns in Sweden, to those leaving school in Norway, and to school entrants in Denmark. Comparing the morbidity rates in those three countries for the decade 1950-1960, it was found that tuberculosis was decreasing among all age groups, but that age-specific decreases were closely associated with vaccination policies in the respective countries. That is, the age-groups given intensive BCG coverage showed a decrease of 20-25 per cent, whereas the nonvaccinated age-groups showed declines of about 10 per cent.

In Hong Kong, where BCG vaccination was given almost exclusively to newborn babies, coverage increased gradually to 71.5 per cent in 1960. From 1954 to 1962 the total morbidity from all forms of tuberculosis decreased by about 80 per cent, but there was only a slow decline in the rate of morbidity in adults (Moodie, 1961 and 1963).

Recent observations in Hungary (Lugosi, 1971), Japan (JATA, 1970), and Birmingham, England (Springett and Sutherland, 1970) indicate a close relationship between vaccination policies and the trend of decreasing morbidity in vaccinated populations.

BCG Vaccine Characteristics

Besides BCG vaccine, other vaccines prepared from attenuated strains of *M. tuberculosis* or other mycobacteria have been recommended for use. Some of them may be as potent as BCG (e.g., vaccine prepared from the vole bacillus, *Mycobacterium microti*). However, BCG is still preferred because no other vaccine has been proved more effective and equally safe.

Variations in the Potency of BCG Strains

The potency of BCG vaccines varies widely from laboratory to laboratory, and vaccines with only 1 per cent culturable particles are no rarity (Guld, 1971). From the various BCG vaccination trials discussed in the preceding section, it appears that in some cases a vaccine which gave very little or no protection and induced only very low, short-lived tuberculin sensitivity was prepared from a strain of questionable potency. This strain later multiplied poorly in laboratory animals and protected them unsatisfactorily when challenged with virulent tubercle bacilli.

Thus, in producing vaccine it would seem reasonable to choose only strains which are fully active metabolically, multiply fast in the host, and yield a vaccine that induces a strong, long-lasting tuberculin sensitivity in children receiving a small standard dose.

Currently, however, the vaccines produced in different laboratories vary widely. Though all existing BCG substrains trace back to the same strain received from Calmette, as one author recently noted, there are "... no two BCG

strains in the world today having identical attributes."[9]

Genetic Changes

Twenty years ago marked differences in BCG strains' immunogenic and sensitizing potency had already been demonstrated (Jensen, 1946; Jacox and Meade, 1949; Dubos *et al.*, 1953). In the course of continuous serial subculture, which was the traditional way to maintain strains, the growth of mutants was unavoidable. In particular, mutants which have a faster growth rate *in vitro* than the mother cells of the strain can quite quickly gain the upper hand. That apparently happened in a number of strains. Sometimes culture media or growth conditions were altered deliberately, with the aim of obtaining a more innocuous strain producing fewer complications or less tuberculin sensitivity; but most of the changes occurred unexpectedly for unknown reasons not subject to control at the time.

Numerous strains have changed their morphological appearance, pigmentation, viability, and growth rate; quite often their protective and sensitizing characteristics have been correspondingly weakened. However, if a strain spontaneously loses the characteristic properties for which it was originally chosen, it is very likely that the strain's mother cells have already been replaced by mutant cells. Such a strain has gone out of control, and its potency is questionable.

The Seed-Lot System

In order to prevent genetic changes and minimize this risk, the traditional way of maintaining strains by serial subculture had to be abandoned. Introduction of the more recent seed-lot system was a significant improvement.

The seed-lot principle consists of preserving a selected strain in dry form at such low temperatures that multiplication cannot occur. At deep-freeze temperatures metabolic requirements are reduced to a minimum. A portion of the bacterial population dies, but a sufficient number of culturable particles can survive for decades. Since no multiplication is taking place, genetic change is eliminated, and so the system accomplishes its goal of preventing such change.

In practice the primary seed-lot is a large batch of freeze-dried BCG stored in ampules. One ampule is opened at a time and the content inoculated into culture media. From this culture subcultures are prepared, until the strain has fully recovered; this takes some two or three passages. Subcultures for preparing either vaccine or a secondary seed-lot are established, but no more than 12 passages are permitted in all. After the twelfth passage, i.e., in about 10-12 weeks, a new ampule must be taken from the primary seed-lot.

This limited number of passages prevents deteriorating changes, because the short reproductive period minimizes the risk of mutation. Now BCG has indeed become the *"virus fixé"* Calmette believed he had obtained. But the limit of twelve passages preceding vaccine preparation (including all preliminary passages) must be strictly adhered to. Actually, it appears advisable to further decrease this limit, providing a wider margin of safety, particularly in laboratories which produce vaccine from a secondary seed-lot. (A secondary seed-lot is prepared at the earliest time possible, e.g., after the third preliminary passage, and preparation of the vaccine requires three more preliminary passages. That means that before vaccine production from a secondary seed-lot can begin, six preliminary passages and sometimes more have already been made.)

The seed-lot system is the most important feature of the "Requirements for Dried BCG Vaccine" adopted by the WHO Expert Committee on Biological Standardization in its Eighteenth Report (1966). Today most production laboratories routinely use the seed-lot system.

Freeze-Dried Vaccines

Because of the great convenience of freeze-dried BCG vaccine, demand for it is steadily

[9]Guld, J. In *International Symposium on BCG Vaccine*, 1971, p. 143.

increasing. Compared to liquid vaccine, the advantages of a heat-stable product in tropical climates are clear. Some of these heat-stable freeze-dried products can be stored at temperatures above 30°C for about a month and will keep in the refrigerator at 4-5°C for a year. Another essential advantage is that any inferior batches can be identified and removed before the vaccine leaves the laboratory, because all tests can be completed before the vaccine is used. Liquid vaccine, because of its short expiration time, must be used before most quality-control test results are available; this sometimes leads to rather embarrassing situations.

Many laboratories producing liquid vaccine hope to embark on production of freeze-dried vaccine. But to prepare a freeze-dried product of satisfactory quality is far more difficult than production of liquid vaccine. Not only does it require a strain which can be freeze-dried without being killed off, but it also demands sophisticated machinery, a complex technology,[10] meticulous and elaborate quality-control procedures, and highly skilled and reliable personnel. These conditions would be very difficult to achieve in all the territories where liquid vaccine has been produced. But even if all the technical obstacles could be overcome, there would still be a long way to go before a semi-industrial production plant could be put into efficient operation. Besides the need to maintain quality standards, such a plant would face manpower difficulties and other managerial problems with a potential for causing repeated production breakdowns. These are the reasons why WHO's warning that "... the multiplication of BCG production centers should be discouraged"[11] must be taken seriously.

In general, small-country investment in the development of freeze-dried BCG vaccine production is hard to justify, even if one only compares the high production costs with the low market price, disregarding the scarce resources necessary for equipment, maintenance, and the like. If it is not possible to make a profit by producing for export, then it is much better and more economical to buy vaccine from accredited production centers. For the most part, developing countries are supplied through UNICEF.

The WHO Quality-Control Service

WHO has always promoted the idea of having a few reliable large-scale production centers, and with this aim in mind has established a service to control the quality of BCG vaccines. Not only is care taken to control the vaccines currently supplied through UNICEF, but any Government or production center can have vaccine samples examined upon request (Document WHO/TB/Technical Guide/6, 1967). The quality control includes a periodic assay in man (Nyboe, 1966), facilitated by the WHO International Reference Center for BCG Seed Lots and Control of BCG products.

During the last few years, since establishment of these services, there has been a marked drop in the large quality variations affecting both liquid and freeze-dried BCG vaccine that used to occur in many laboratories.

BCG Vaccination Techniques

Of the three most common techniques (intradermal injection, percutaneous scarification or multipuncture, and peroral application) the intradermal technique has been generally acknowledged as the most precise. By using

[10] After the bacterial mass is harvested from the medium on the appropriate day of growth, and subsequently filtered and pressed, the semi-dry material is mechanically homogenized according to exacting standards. It is then diluted, put into ampules, and freeze-dried. Eventually the ampules are sealed in a near-perfect vacuum. During the whole procedure the product must be strictly protected against exposure to sunlight and kept in rooms with pressurized, filtered, and sterile air. It is thus difficult, though essential, to keep all the production factors which determine the viability and heat stability of the vaccine under safe control.

[11] *Vaccination against Tuberculosis; Sixth Report of the Expert Committee on Tuberculosis.* WHO Technical Report Series, No. 88, 1954, p.4. See also *WHO Expert Committee on Tuberculosis, Eighth Report,* WHO Technical Report Series, No. 290, Geneva, 1964.

leak-proof syringes, the vaccine dose can be kept fairly accurate and thus the risk of complications due to overdosage is low. The intradermal technique also permits the use of a much more dilute vaccine than percutaneous methods, which use vaccines containing concentrations of culturable particles 20 to 200 times higher; concentrations required for oral vaccines may be even greater.

Except with the intracutaneous technique, one cannot determine how much vaccine is actually entering the body. Therefore, a wider range of dosages, and more underdoses and overdoses as well, must be expected. Overdoses are likely to cause undesired reactions, and in order to reduce the risk of them the average vaccine concentration must be lowered. As a result the proportion of complications will be lower, but the risk of underimmunization will be higher.

Various multipuncture techniques use either automatic pistol-type (spring-driven) devices or simple discs made of steel or a disposable material. They have been advocated as being simpler and faster, enabling unskilled staff members with no special training to vaccinate large population groups.

The multipuncture technique might occasionally give satisfactory though inferior results in comparison with intradermal techniques. But experience indicating this is derived mostly from research or special studies. Whether the technique is actually robust enough to give equally good results under practical and ordinarily less favorable conditions is not known; at least, no evidence that it can do so is yet available.

A WHO-assisted study is presently examining a multipuncture technique using the bifurcated needle commonly employed in smallpox vaccination. When failures are found to be within tolerable limits, field trials under real-life conditions will be undertaken. Provided the results are still acceptable, the bifurcated needle technique might be recommended for use in situations where the alternative would be too little vaccination or none at all. An operational advantage of this technique in countries where smallpox vaccination is a permanent policy is that BCG vaccinations could be given by smallpox vaccinators without special training. This would permit both vaccinations to be applied at the same time and with the same technique.

One intradermal technique which seemed rather promising uses a jet-injector based on the air-gun principle. Various types that generate the necessary air pressure can be operated by hand, foot, or an electric current. A supposedly uniform dose is shot into the superficial layers of the skin, as with the intradermal syringe-needle technique.

A number of studies have compared the various types of jet-injectors with the intradermal syringe technique, and a thorough analysis of these studies was recently published (Dam *et al.*, 1970). Surprisingly, these studies failed to demonstrate the precision one would expect of an automatic apparatus. The size of post-vaccination tuberculin reactions was smaller, on the average, though the variations were similar to those of the syringe technique. However, the variation in lesion size was much larger, indicating individual differences in the way the jet-injector dispenses vaccine into the superficial layers of the skin. In order to achieve post-vaccinal tuberculin reactions of the same size, 50-250 per cent higher doses of vaccine had to be given, depending on the type of jet-injector used. However, the lesions produced by these higher doses were substantially larger than those resulting from syringe injection.

Disregarding the operational vulnerability and cost of jet-injectors, they offer an advantage only in situations where large numbers of eligible persons can be lined up, and where means of repairing the equipment is always at hand. Another difficulty arises when adults and infants have to be vaccinated in alternating sequence–and when infants are to be given a smaller dose–for it is not technically feasible to adjust the jet-injector instantly.

Oral vaccination, the original technique recommended by Calmette, has been abandoned by a number of countries where it had been

used routinely, despite its simplicity. Although sufficient evidence is still lacking, it is believed that it might offer an efficient way of vaccinating newborns. Critical studies in older age groups, however, revealed that oral vaccination did not fulfill its expectations. Because of the high vaccine dosages required, the risk of serious complications for newborn infants appeared to be rather high. Frequent cervical lymphadenitis and BCG infection of the middle ear with subsequent impairment of hearing were among the reasons why oral vaccination has been rejected in the past.

Direct BCG Vaccination

Indiscriminate BCG vaccination, i.e., without prior tuberculin testing, has been thoroughly investigated by several WHO projects in various parts of the world. Also, after the WHO Expert Committee on Tuberculosis (1964) recommended direct BCG vaccination as a country-wide policy, a number of further studies examined the risk of undesired effects. Among other things, the incidence of lymph node enlargement was investigated; no difference was found between reactors and non-reactors (Chavganc *et al.*, 1969; Document WHO/TB/58, 1967). Other studies dealt with the hypothetical risk of reactivating quiescent or healed tuberculosis in children (Egsmose, 1969), and the possible deteriorating effect that vaccination might have on specific lung lesions in persons undergoing radiological and bacteriological follow-up (Gothi *et al.*, 1964). No adverse effect of direct BCG has been reported in any of the studies.

Since direct BCG vaccination has been shown highly acceptable in many countries, it could be adopted as a national policy. Its obvious operational advantages, which result in higher outputs while almost halving the workload, makes direct BCG vaccination recommendable, particularly in countries where health personnel shortages are a serious constraint.

Simultaneous or Combined Administration of BCG and Other Vaccines

Whereas direct BCG vaccination had been accepted by national authorities with certain caution, and only after pilot studies had proven its innocuity, the idea of simultaneous BCG and smallpox vaccination was welcomed with almost no hesitation. It has been found experimentally in animals (Kawazaki, 1959), and by tests in children (Moodie, 1963; Lin, 1965, 1966; Christensen, 1966; Baily, 1967) that there is no interference with respect to development of local lesions, the take rate, or tuberculin sensitivity. Convenience for the public, as well as for health authorities and vaccinators, has led several countries to adopt simultaneous smallpox and BCG vaccination of children as a national policy. The recent finding that a potent smallpox vaccine given to infants can protect them for some six years or more, and that revaccination at primary school age may extend the protective effect for two decades or even longer (Henderson, 1971) is very promising and supports the idea of pooling both vaccination programs.

Other investigators have made pilot studies of simultaneous application of BCG with other vaccines, including vaccines for measles (Dutertre, 1971) and yellow fever (Chambon, *et al.*, 1971).

In a few studies BCG and smallpox vaccines have been combined in one injection; the possibilities for a combined BCG-yellow fever injection have also been examined. No adverse immunological interference nor any other untoward effects were observed (Heyworth, 1970; Chambon, 1971). However, the degree to which the mixing of vaccines by field workers is a safe procedure needs to be investigated. Also, the preparation of mixed vaccines in one ampule should be studied. Although immunologists tend to favor giving many vaccinations together with BCG to as many people as possible in the shortest possible time (Labusquiere, 1972), further research will have to produce firm evidence that this practice is harmless and

useful before it can be recommended as national policy (Dahlström, 1972).

BCG Vaccination Against Diseases Other Than Tuberculosis

Leprosy

Some striking similarities between leprosy and tuberculosis, primarily the fact that leprosy is caused by an alcohol and acid-fast mycobacterium, have given rise to three controlled field trials (WHO Expert Committee on Leprosy, 1970). The first was initiated in Uganda in 1960; it involved 18,000 children 0-15 years old, who were relatives of leprosy patients or were living in contact with them. Another trial was conducted in Burma in 1964, utilizing 27,000 children 0-14 years old in a high-prevalence area; and a smaller trial was carried out in New Guinea using 5,000 persons of all ages, who were also living in a high-prevalence area (see Table 4). Whereas in Uganda an incidence reduction of 82 per cent could be observed, this figure was only 55 per cent in New Guinea, and in Burma no clear protective effect was observed. The Committee concluded that the differences between these results should first be studied, and considered it premature to recommend BCG vaccination for prevention of leprosy.

Leukemia

Several years ago it was reported that BCG might help protect against leukemia or might have some influence on its clinical development (Mathé *et al.*, 1967). A recent report from Canada has supported this observation (Davignon, *et al.*, 1970, 1971). In Quebec Province the frequency of leukemia in children up to five years old was found to be only half as high in children vaccinated with BCG at birth as it was in unvaccinated children.

The last report on the Medical Research Council's vaccination trial (1972) in Great Britain, which evaluated the results of 15 years of observation, found the mortality from neoplasms of lymphatic and hematopoietic tissues for BCG-vaccinated persons between the ages of 15 and 30 to be 2.4/100,000 per year, while for unvaccinated persons of the same age the rate was 4.1/100,000. Although the total number of persons studied was small and the difference was not statistically significant, the findings were on the same order of magnitude as those from Canada and thus supported them. In a similar analysis (Comstock *et al.*, 1970, as quoted in the BMRC report) no indication was found that BCG might prevent or promote the development of leukemia.

BCG Revaccination

It must be said that regrettably little is

TABLE 4–Studies of BCG vaccination against leprosy (preliminary results).

Study	Administration of BCG vaccine	No. of persons	No. of cases	Per cent reduction of cases	Years of observation
Uganda	Unvaccinated	9,052	179		6+
	Vaccinated	9,036	32	82	
Burma	Unvaccinated	13,780	264		5.5
	Vaccinated	13,797	224	15	
New Guinea	Unvaccinated	2,296	18		4.5
	Vaccinated	2,318	8	55	

Source: World Health Organization. *WHO Expert Committee on Leprosy, Fourth Report*, Geneva, 1970.

known about the protective efficacy of revaccination with BCG in man. No controlled trials have been conducted except in animals. One of these animal studies (Tolderlund *et al.*, 1967) investigated the duration of immunity and tuberculin sensitivity in guinea pigs revaccinated with BCG and observed for five years. It was found that animals vaccinated shortly before challenge, irrespective of whether it was their first vaccination or a revaccination, survived longer than animals vaccinated only once and challenged after a long interval. This finding could conceivably imply that revaccination might be justified in situations or areas where the risk of disease is high due to frequent exposure to infection.

Other findings of this animal study were more conclusive; e.g., it was found that the protective effect of BCG vaccination lasted throughout the animal's life-span. Although the effect tended to decrease, it could be demonstrated in even the oldest animals (which were kept for an average of five years). Waning protection could also be observed in man after 10 years of the BMRC vaccination trial, though the decrease was less pronounced. It may thus be reasonable to conclude that children living in a high-prevalence environment who are vaccinated during infancy or early childhood could benefit from revaccination after a 10-year interval, i.e., at about 12-15 years of age.

The same study demonstrated that BCG-induced tuberculin sensitivity can eventually disappear even though protection remains—provided no tuberculin testing is done in the interim. One group that was repeatedly given tuberculin tests showed no lessening of tuberculin sensitivity. Nevertheless, the BCG-conferred protection was not influenced by the frequency of the tuberculin test.

The study also confirmed that a single tuberculin test is capable of restoring weakened postvaccination tuberculin sensitivity to the degree that it was originally found in the group tested repeatedly. As has been previously indicated, repeated testing can only maintain BCG-induced sensitivity, but cannot maintain protection or increase it.

The restoration of a waned BCG-induced tuberculin sensitivity in man by means of a single injection of tuberculin has been demonstrated by several investigators (Magnus and Edwards, 1955; Ferebee and Mount, 1963; Narain *et al.*, 1966; Guld *et al.*, 1968). From these studies it can be concluded that increases and decreases in BCG-induced tuberculin sensitivity have no correlation with variations in BCG-conferred resistance against tuberculosis. There is no scientific basis for the very common practices of revaccinating persons whose tuberculin skin sensitivity has waned and denying revaccination to those who still react to tuberculin, particularly if the latter have regularly been retested.

Thus the decision about what revaccination intervals have to be chosen cannot be based on the duration of postvaccination tuberculin sensitivity, but only on evidence from controlled trials in man. From those trials in which significant BCG protection could be observed, there is little evidence that it would last much beyond 10 years. Thus, in high-prevalence areas a ten-year revaccination interval seems sound. To revaccinate at three-year intervals, as is the policy in some moderate-prevalence countries, is certainly not justified when a potent vaccine is being used. If the potency of the vaccine in use is uncertain, then instead of reducing the intervals it is better to replace the vaccine with one known to have adequate strength. Exceptions, i. e., candidates for early revaccination, might include persons (such as newborn babies) who were deliberately given a weak dose, or those who received vaccine from a poor-quality batch. Naturally a revaccination policy should not be introduced into a country's programs until the vast majority of the eligible population has received a primary vaccination.

BCG-Induced Tuberculin Sensitivity

A series of systematic studies conducted 20 years ago (Edwards *et al.*, 1953) demonstrated that skin sensitivity to tuberculin caused by infection or vaccination is generally not a qualitative attribute which is either present or

absent; nor should it be classified positive or negative (as is often done). It was clearly shown that tuberculin sensitivity is a phenomenon which can only be described adequately in quantitative terms, e.g., the size of a skin reaction measured in millimeters.

This conclusion applies particularly to BCG-induced tuberculin sensitivity. It has been convincingly demonstrated that a group of individuals who were tuberculin nonreactors before they were vaccinated with a uniform vaccine acquired sensitivity which showed a unimodal pattern; i.e., the sizes of skin reactions could be grouped around a mean conforming to a normal frequency distribution. It would be meaningless to divide such an obviously homogeneous group and call those with a small reaction negatives and the rest positives or converters. Even when a weak dose of BCG has been given, this unimodal pattern of size distribution can be demonstrated by administering a stronger dose of tuberculin.

It is therefore unjustified to separate out the proportion of reactions above a certain limit and to declare that this proportion represents the conversion rate. It is sounder to grade postvaccination sensitivity by a simple measurement, such as the average size of skin reactions (in millimeters) to a specified dose of tuberculin.

It should be noted that prevaccination sensitivity provides information about a certain individual or group, while postvaccination sensitivity provides information relating primarily to the vaccination applied.

Assessment Procedures

With the introduction of freeze-dried vaccine, batch-to-batch variations have practically been eliminated. Increased heat-stability, as well as the practice of dispensing vaccine in ampules of dark glass, is eliminating gross deficiencies due to improper handling. Thus, the use of the tuberculin test for assessment of vaccine efficacy has become less important. Assessment is therefore directed primarily at determining coverage, which is another index of program efficiency. This entails the inspection and counting of local lesions or scars. The work is thus rather simple and requires no special skill (Mokthari, Rouillon, and Dam, 1970); but it can provide the desired information quite fast, so that timely corrective action can be taken.

SUMMARY

Despite a great fund of experience and accumulated knowledge, the subject of BCG vaccination remains controversial. This presentation attempts to give a balanced account of the questions involved, and to focus on findings that can provide a scientific basis for framing national policies.

The most valuable data on BCG efficacy has come from controlled trials. Analysis of these trials indicates that low levels of efficacy achieved in some instances resulted from use of poor-quality vaccine. Where it appears certain that good-quality BCG was used, the results show that vaccination was effective in preventing the disease.

A major problem with the bacterial cultures used in BCG production is that mutants can take over a culture, occasionally depriving it of the ability to confer protection against tuberculosis. Freeze-dried vaccine produced by the seed-lot method greatly reduces the risk of mutation and offers other important advantages as well. However, since the production process is quite sophisticated and requires elaborate quality-control procedures, the number of production centers should be limited.

Of the various available methods for administering BCG vaccine, the intradermal technique is generally conceded to be the most precise. Multipuncture and oral vaccination techniques

have also been used. A WHO-assisted study is currently examining the effectiveness of a multipuncture technique using the bifurcated needle often employed in smallpox vaccination.

Direct BCG vaccination (that is, vaccination without prior tuberculin testing) has been studied thoroughly, and no adverse effects have been reported. Also, there may be definite advantages in simultaneous administration of BCG and other vaccines–notably smallpox vaccine–though actual mixing of BCG and other vaccines in the same injection is a matter that requires further investigation.

Not enough is yet known about the protective efficacy of BCG revaccination in man, though beneficial effects have been demonstrated in animal studies. Human vaccination trials in which significant BCG protection was observed give little indication that such protection lasts much over 10 years. There thus appears to be a sound basis for revaccination at 10-year intervals in high-prevalence areas.

Controlled field trials indicate BCG may also confer protection against leprosy, though widely differing results show a need for additional study of this subject.

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