CHAPTER 10

GENERAL SUMMARY

This monograph sets out the results of nearly three years' intensive study of BCG vaccination in schoolchildren, whose response to vaccination was gauged by the level of tuberculin allergy and by the cutaneous reaction at the site of vaccination. The work was done in connexion with existing BCG-vaccination programmes in schools in Denmark and several other countries. It differed from the ordinary vaccination programmes mainly in the care taken to make uniform, accurate, and unbiased observations, and the fact that the vaccines and ways of vaccinating were *deliberately* varied. The subjects studied included the effects of long-continued storage, heat, light, changes in the technique of intracutaneous vaccination, variations in the preparation and concentration of the vaccine, mixtures of living and dead bacilli in different proportions, and the variability of vaccines prepared by different producers.

Altogether more than 40,000 children in Denmark, Mexico, Egypt, and India, most of them 7 to 14 years of age, were given pre-vaccination tuberculin tests, on the basis of which some 23,000 were vaccinated. By statistical-sampling techniques, the vaccinated population was divided into nearly 300 groups, most of which contained 50 to 150 children. Each group differed from the others in respect of some planned variation in the vaccine used or in the method of vaccinating. Post-vaccination examinations 2-3 months, one year, and two years later included measurement of the transverse diameter of the indurated area of the reactions to an intracutaneous tuberculin test, measurement of the local reaction (or scar) at the site of vaccination, and observation of local complications of the vaccination.

The work has yielded important information about tuberculin allergy—both the allergy observed in children before vaccination, and the allergy induced by BCG. Perhaps because of observations in animals, sensitivity to tuberculin has long been thought of in qualitative terms—as something the body does or does not possess. Yet almost every chapter in this report clearly shows that tuberculin sensitivity is *quantitative*, and not a simple qualitative characteristic that may be described as present or absent, positive or negative. But quantitative measurement may reveal qualitative differences. This is well exemplified by the pre-vaccination tuberculin reactions of children living in different countries. From the distributions

of the measured sizes of such tuberculin reactions, we can conclude that there must be at least two different kinds of accidentally acquired sensitivity to tuberculin. One kind, found everywhere, is manifested by a strong reaction to a weak dose of tuberculin; this high-grade sensitivity is undoubtedly caused by infection with virulent tubercle bacilli. The other kind, not found everywhere, is reflected in smaller reactions to the same weak dose, and is generally designated as " non-specific " ; it is apparently unrelated to tuberculous infection. This " non-specific " sensitivity, whose cause is still unknown, was disclosed primarily because tuberculin reactions were measured and because data were collected in a uniform way in different areas.

The view that tuberculin allergy cannot be adequately described by the " percentage of positive reactors " is also supported by studies of tuberculin sensitivity induced by BCG. Children given the same vaccine generally respond to vaccination in much the same way: their tuberculin reactions are distributed in a unimodal pattern roughly conforming to a " normal " frequency curve. Some reactions are smaller than the average, some larger; but the group as a whole shows a single biological response. And this has been found to be the case whatever the *level* of allergy produced. Following the mass BCG-campaigns, for example, the level of post-vaccination allergy of some groups was high, for others it was very low; even greater variations in allergy were produced by the experimental vaccines used in these studies; yet the tuberculin reactions of each group were distributed in a unimodal pattern. Postvaccination allergy, we believe, can best be expressed by a simple, stable, and more informative figure—the average size of the tuberculin reactions of the group. This does not involve complex statistical procedures, and enables us to discriminate differences in the level of allergy produced by vaccination.

Attempts to determine quantitatively the difference between vaccines in routine use from the same and different laboratories have not been very successful, partly because it was not possible to control many of the factors known to influence the measurements, partly because there is no stable standard product as a point of reference, and partly because of qualitative differences between vaccines. It may be estimated, however, that some batches of Danish vaccine could have been diluted ten times and still have produced the same allergy in children as other batches used in full strength. Between a few samples of vaccine from six other BCG production laboratories, the potency varied more than between samples of Danish vaccine.

The average sizes of post-vaccination tuberculin reactions and vaccination lesions regularly decreased with reduction in the number of BCG organisms per dose. But this decrease was much less than was expected: the effect of doubling or halving the number of organisms was hardly

detectable. With a tenfold reduction in dose, the size of the tuberculin reactions was reduced by 2-4 mm ; and for some regular batches of vaccine the dose of BCG could be reduced several hundred times without impairing the result as judged by some criteria for " positive " reactions in general use today. As regards tuberculin allergy in vaccinated children, it is clear that small differences in the amount of BCG injected has little practical importance.

The inclusion of dead BCG organisms in a vaccine also gave unexpected results. Completely dead vaccine produced a low level of allergy, but the addition of only a small fraction of living organisms made the vaccine surprisingly potent. Apparently, the presence of living BCG augments the effect of the dead organisms, or vice versa, so that the allergy produced is greater than would be expected from the sum of the two component elements acting separately.

Many different studies were undertaken to determine the effect of exposing BCG vaccine to light and moderate heat. The results, while underlining the need for further study, reveal the basic pattern of the reduction of potency of vaccine produced by both light and heat. So long as BCG vaccine is protected from light and kept at near-freezing temperatures, potency is lost very slowly—the loss is measurable only over a period of months. As the storage temperature is raised, the rate of loss increases : at 20°C, loss of potency is detectable within a few weeks. Light has a similar injurious effect, but it acts much more quickly ; indirect daylight produces measurable effects in a matter of hours, and direct sunlight in minutes or even seconds. These findings, though not basically new, show that failure to protect vaccine from exposure to high temperatures or to light, for even short periods, undoubtedly results in the widespread use of vaccine which, though fresh in a time sense, has lost much of its potency for producing tuberculin allergy.

Several studies were designed to determine the significance of variations in the technique of intracutaneous injection of vaccine. So far as post-vaccination allergy is concerned, it makes little difference whether the vaccine is injected into the most superficial layers of the skin, as is recommended, or into the subcutaneous tissues ; nor whether the volume injected ranges from one-half to as much as three times the recommended 0.1-ml dose. On the other hand, both the size and the severity of the local lesion are greatly influenced by the depth of injection : the deeper the injection, the larger the local lesion. This finding, together with those from studies on dead BCG, suggests that some " good strong takes " at the site of vaccination may be the result of injecting into the deeper layers of the skin (or subcutaneously) a vaccine containing a high proportion of dead organisms.

The report contains no account of concurrent laboratory work. When the field work was begun, it was thought that the usual routine laboratory observations would suffice to supplement the field work. Later, however, it was found that the colony counts made from the vaccines varied widely, often without any apparent relation to changes in the technique of preparing the vaccine ; and the animal work was based on too few animals to show any except gross changes in the potency of the vaccine. It is now known that the colony counts were probably influenced to a large extent by an extraneous source of variation : in preparing and culturing material for counts, the exposure to light in the laboratory had varied greatly. Inability to use the results of the laboratory tests for comparison with the field results on children, while regrettable, is somewhat offset by what has been learned of the pitfalls in drawing conclusions from laboratory work without concomitant field observations. Laboratory control and check of BCG vaccine is necessary but not sufficient : the decisive test is the response in man.

The study of immunity produced by BCG vaccination is, of course, most important and urgent. But, at the present time, immunity can be assessed only on large groups of people by long-term control studies, in which some are vaccinated and others not. Allergy, on the other hand, is a readily available yardstick for measuring the immediate effect of vaccination in an individual person at any point in time. The allergic response is so apparently a protective phenomenon that, in the absence of a direct measure of immunity, tuberculin allergy warrants intensive study as a guide, however imperfect, to the effectiveness of BCG vaccination.