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EXPERIMENTAL STUDIES OF VACCINATION, ALLERGY, AND IMMUNITY IN TUBERCULOSIS

2. Effect of Varying the Dose of BCG

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SYNOPSIS

Results are given for one of a series of projects designed to investigate the relation between observable post-vaccination responses and acquired resistance to tuberculosis. Controlled variations in the dose of BCG vaccine have previously been shown to cause systematic variations in the degree of skin sensitivity to tuberculin and the size of vaccinal lesions in human beings : the purpose of the present project was to see if similar variations would be produced in guinea-pigs and then, by infecting the animals with virulent tubercle bacilli, to see how survival time correlates with tuberculin allergy and vaccinal lesions.

Four doses of freshly prepared BCG vaccine, ranging from 1/100 to 10 times the dose ordinarily used for intradermal vaccination of humans, and one dose of heat-killed BCG 100 times that strength, were used to vaccinate five groups of guinea-pigs, each containing 120 animals. A sixth group of 120 animals was not vaccinated. All animals were tuberculin-tested just before and five weeks after vaccination, challenged with a strong dose of H-37 Rv, and then allowed to die, so that survival time could be used as a measure of resistance.

As the dose of living BCG was increased, groups of guinea-pigs showed a progressive increase in the average degree of post-vaccination tuberculin allergy, size of vaccinal lesion, and length of survival after virulent infection. The heat-killed BCG resulted in weak allergy and a short survival time, yet the vaccinal lesions averaged about as large as would be expected from a corresponding dose of living BCG. These results (excluding studies of survival time) correspond closely to those found in human studies.

The implications of the results with respect to practical BCG vaccination programmes, while no more than speculative at present, point toward possible advantages in inducing high degrees of tuberculin allergy and toward the dubious significance of the vaccinal lesion as an index of a vaccine's immunizing potency.

* This paper reports results obtained in part (Project III) of the laboratory research programme jointly directed by Carroll E. Palmer and Shirley H. Ferebee of the United States Public Health Service, Sven Nissen Meyer of the WHO Tuberculosis Research Office, and Hubert Bloch of the Public Health Research Institute of the City of New York.

In the laboratory, Helen X. Gertz was in charge of carrying out the daily activities in accordance with the study plan. Tuberculin-testing and vaccination were done by Lillian Lang, and the statistical operations were supervised by Louise Hopwood. Knud Tolderlund of the Statens Seruminstitut, Copenhagen, prepared the BCG vaccines for the project and made the bacterial counts. Sven Nissen Meyer and Carroll E. Palmer were mainly responsible, with the assistance of Phyllis Q. Edwards, for the preparation of the present report.

The first paper in this series (see page 13) described the rationale and general features of a research programme undertaken in laboratory animals in an attempt to find practical guide-lines for judging, by analogy, the results of BCG vaccination in man. The programme consists of a series of projects, each designed to vary some relevant factor (or factors) in vaccination and then, by challenging the animals with virulent tubercle bacilli, to examine the relation between observable responses to vaccination and acquired resistance to tuberculosis.

Among the many factors that may influence the results of vaccination, the dose and composition of the vaccine have probably received the most attention both in the laboratory and in studies on human populations (Ebina et al.,[6] Frappier et al.,[9] Giovanardi & Grosso,[11] Jensen,[15] Murohashi [17]). Vaccine may be subjected to controlled variations in a number of ways, one of the simplest being to vary the dose without changing the composition. In large-scale studies on human beings (Edwards et al.[7]), the dose of ordinary living BCG was shown to have a direct effect on the degree of post-vaccination allergy : almost any average level of allergy could be produced by vaccinating groups of persons with intradermal doses ranging from 1/10,000 to a fourfold concentration of the ordinary strength. Variations in dose similarly affected the average size of the vaccinal lesions.

Dosage was therefore one of the first factors to be deliberately varied in the present research programme. The purpose of the project reported here was to see if a wide range of doses of BCG, similar to those used in the human field studies, would cause the same kind of variations in the level of post-vaccination allergy (and size of vaccinal lesions) in animals; then, by challenging the animals with virulent tubercle bacilli, to measure their resistance in terms of survival time.

Material and Methods

The experiment was carried out in accordance with the general principles described in the previous paper. The following description of materials and technical procedures will therefore be limited to a brief outline, supplemented by details that pertain specifically to the present project.

Vaccines

The five different strengths of vaccine used in the experiment were prepared from the same batch of BCG, number 1032, harvested on 9 January 1953, at the Statens Seruminstitut, Copenhagen. This was one of the regular weekly batches prepared by Tolderlund according to his routine method for intradermal vaccination of human beings. The different strengths were made by direct dilutions from a stock suspension containing 75 mg moist-weight bacilli per ml. The strength used for human vaccination, containing

0.75 mg moist-weight bacilli per ml, is referred to as " standard strength ". Cultures of the standard strength suspension of batch 1032 in Löwenstein tubes showed 4×10^6 viable bacillary units per dose (0.1 ml).

The following vaccines were used :

Living BCG, diluted to 1/100 standard strength (1/100 st.);
Living BCG, diluted to 1/10 standard strength (1/10 st.);
Living BCG, standard strength (1 st.);
Living BCG, 10 times standard strength (10 st.);
Heat-killed BCG, 100 times standard strength (100 st.).

The heat-killed vaccine was included to obtain preliminary information on the effect of using dead BCG, a subject investigated further in later projects. It was prepared by placing a portion of the stock suspension in 10-ml sealed ampoules and immersing them in a water bath at 70°C for 45 minutes. No bacterial growth was observed on cultures of the undiluted (100 st.) heated vaccine.

Animals and procedures

A total of 720 random-bred guinea-pigs were used for the experiment —120 for vaccination with each of the five vaccines and 120 for the control (unvaccinated) group.

Allocation to the six experimental groups was made by placing six animals of the same sex, and with similar hair colour and body-weight, in each of 120 cages. Within each cage, the animals were then allocated at random to the control group or one of the five vaccine groups. Finally, so that the possibility of carrying out later investigations on the effect of sex, weight, etc., should not be precluded, the cages were distributed at random throughout the animal house.

Some of the 120 animals in each of the six groups were used for two pilot studies. All tuberculin tests were omitted in 20 animals from each group to see whether tuberculin-testing affects resistance. Twenty other animals from each of the five vaccinated groups were revaccinated 10 days after the first vaccination, each being given the same vaccine as before. The 220 animals for the two pilot studies were distributed uniformly in all cages and selected at random from the five vaccine groups.[a] The revaccinated animals

[a] The selection of animals for these two pilot studies was made in the following way : 20 cages were selected at random for revaccination with 1/100 standard strength of living BCG, 20 other cages for revaccination with 1/10 standard strength of living BCG, etc., using 20 new cages for each of the five vaccines. Thus only one animal in each of 100 cages was revaccinated, and 20 cages contained animals vaccinated once only.

The animals to be exempted from tuberculin tests were then selected as follows. Each of the five groups of 20 cages used for revaccination was subdivided at random into five groups of four cages. In one of these five subdivisions (composed of four cages), tuberculin tests were omitted for the nonvaccinated (control) animals. Tuberculin-testing was omitted for the animals in the four other subdivisions corresponding to the four vaccines used only once (tuberculin tests were not omitted for animals vaccinated twice). The last group of 20 cages, containing animals in which no revaccination was done, was also subdivided into five groups of four cages, tuberculin tests being omitted for all five vaccinated groups—four cages for each vaccine—but not for the controls.

are excluded from the present report, those exempted from tuberculin tests are included in the analysis of vaccinal lesions and in the survival curves (as they did not differ significantly from the others), but they cannot, of course, be used for the study of allergy.

Pre-vaccination tuberculin tests were given intradermally in the right upper quadrant of the abdomen with 0.002 mg (100 TU) of PPD-S in 0.1 ml of diluent and read 48 hours later.

Vaccines were given 3 days after the reading of the tuberculin tests by injecting, intradermally, 0.1 ml of the specified preparation in the right lower quadrant of the abdomen.

Readings of vaccinal lesions included measurement of the largest diameter of induration at 4, 10, 14, 20, and 24 days after vaccination.

Post-vaccination tuberculin tests were given 32 days after vaccination by injecting 0.002 mg of PPD-S intradermally in both the left upper and the left lower abdominal quadrants. The largest diameters of erythema and of induration were measured after 24 and 48 hours.

Challenge infection. All animals were infected 35 days after vaccination with a liquid Tween-albumin culture of the H-37 Rv strain (from the Trudeau Laboratory, Saranac Lake, N.Y.) by intraperitoneal injection, just below and to the left of the umbilicus, of 0.5 ml of a suspension containing approximately 5×10^6 bacterial units per ml (as determined by plate counts).

Post-challenge tuberculin tests were given 29 days after the challenge infection. The tests were given intradermally in both the left and right upper quadrants of the abdomen with 0.002 mg of PPD-S; the reactions were read after 24 and 48 hours (results are not given in this report).

For technical reasons (infestation with lice) the experiment had to be stopped by killing all surviving animals 9½ weeks after the challenge infection.

Results

Results of the experiment are given in seven figures showing the effects of the different vaccines on vaccinal lesions, tuberculin allergy, and survival time after challenge infection ; two additional figures show the interrelation between these measures of response.

Fig. 1 shows frequency distributions of the vaccinal lesions by size of induration as measured 10 days after vaccination. With increasing strength of BCG there was a gradual increase in the average size of the lesions, from no lesion for most of the group vaccinated with the most dilute

FIG. 1. FREQUENCY DISTRIBUTIONS OF SIZE OF VACCINAL LESIONS, 10 DAYS AFTER VACCINATION, FOR GROUPS OF GUINEA-PIGS VACCINATED WITH DIFFERENT STRENGTHS OF BCG VACCINE

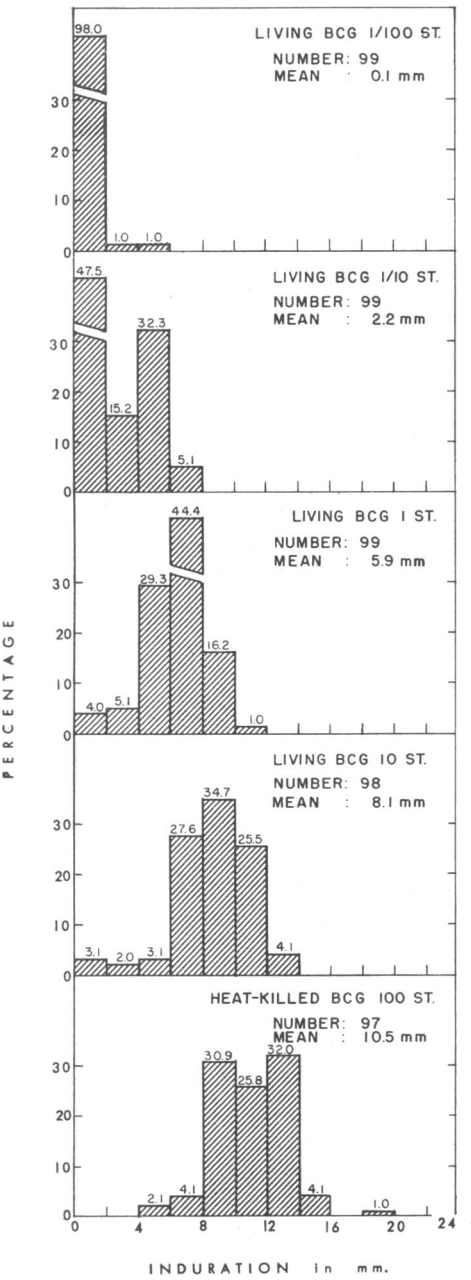


FIG. 2.
MEAN SIZE OF VACCINAL LESIONS, 10 DAYS AFTER VACCINATION, FOR GROUPS OF GUINEA-PIGS, ACCORDING TO STRENGTH OF BCG VACCINE

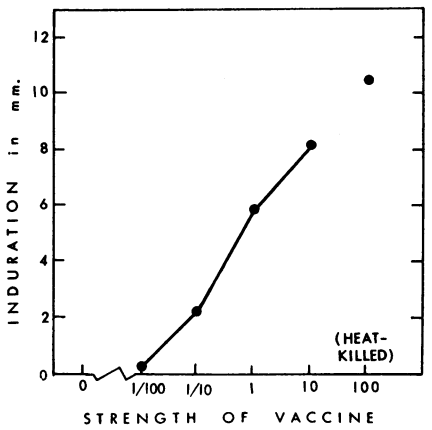
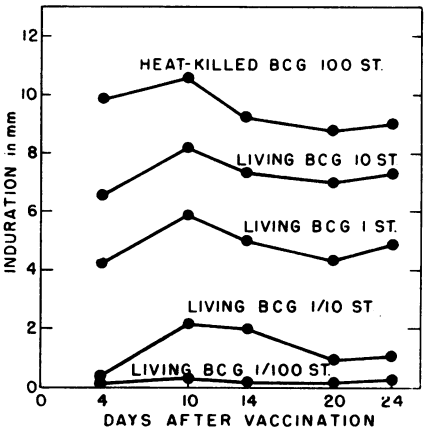


FIG. 3.
MEAN SIZE OF VACCINAL LESIONS AT DIFFERENT TIMES AFTER VACCINATION, FOR GROUPS OF GUINEA-PIGS VACCINATED WITH DIFFERENT STRENGTHS OF BCG VACCINE

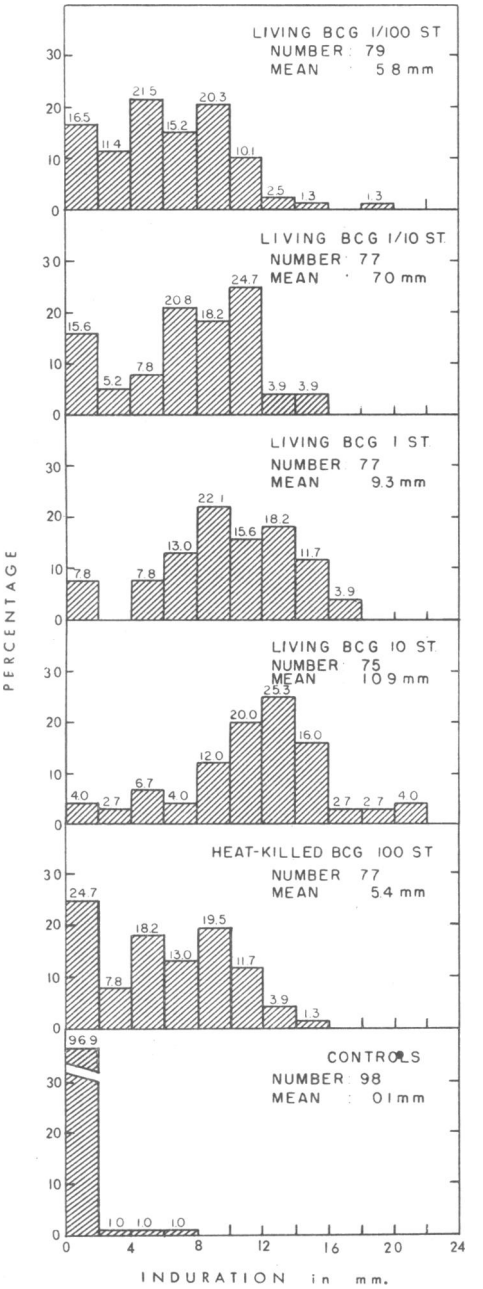


vaccine to lesions averaging 8.1 mm in diameter for the vaccine 10 times the standard strength. Killed BCG 100 times the standard strength caused the largest lesions; in fact, they were about the size one might have expected from the same strength of living vaccine.

The shapes of the histograms suggest that the simple arithmetic average or mean will serve, just as it does for results in human beings, as a numerical description of the lesions produced by different vaccines. The increase in average size of lesions with increase in dose of BCG is clearly brought out by fig. 2, where the strength of vaccine is plotted logarithmically on the abscissa, and the mean size of the lesions on the ordinate. There is apparently a linear relation between the two, with the lesions increasing by an average of about 2½ mm for each tenfold increase in dose. The point representing the killed vaccine falls on or near a continuation of the line for the different dilutions of living BCG. There is no indication that doses of vaccine greater than the standard strength tend to produce alarmingly large vaccinal lesions.

Fig. 3 shows the mean sizes of vaccinal lesions measured at five different times during the first month after vaccination. The variations in size with time are relatively small and the maximum at the tenth day must be taken with some reservation : no matter how experienced and careful an observer may be, his observations cannot be expected to remain constant over a period of time. Comparisons from group to group may, however, be made with confidence at any one observation period, because the lesions were read on animals taken in a random sequence from all groups and with no possibility of the reader's having known any animal's group or previous

FIG. 4. FREQUENCY DISTRIBUTIONS OF SIZE OF TUBERCULIN REACTIONS, 32 DAYS AFTER VACCINATION, FOR GROUPS OF GUINEA-PIGS VACCINATED WITH DIFFERENT STRENGTHS OF BCG VACCINE



reaction size. It thus appears that the relation between dose of BCG and mean size of vaccinal lesion changed very little during the first month after vaccination. At no time did more than a very few of the animals vaccinated with the 1/100 st. vaccine show a measurable lesion. The most concentrated vaccine produced lesions that were consistently the largest, about 10 mm in diameter, despite the absence of living bacilli.

In fig. 4 the animals from the control and the five vaccine groups have been distributed by size of tuberculin reactions 32 days after vaccination. The results are for tests given in the left lower quadrant of the abdomen and read at 48 hours. As shown by the lowest histogram, most of the 98 animals in the control group had no reaction, although small reactions were recorded for about 3% of them. As no measurable reaction was recorded for any of the animals on the pre-vaccination tests, these results for the controls must be ascribed either to unkown sources of sensitization or, more likely, to observational or even clerical errors. The frequency of such errors would probably have been lower had the reader (or the clerk, or both) known what results to expect, as they unavoidably did in reading the pre-vaccination tests, and where none of the animals were recorded as having a measurable tuberculin reaction. If, however, they had been able to identify the animals belonging to different groups when reading the post-vaccination tests, the results could have been influenced, perhaps considerably, by the element of personal bias.

As shown by the first histogram in fig. 4, demonstrable allergy was produced by the weakest dilution of living vaccine : the mean size of the reactions measured 5.8 mm as compared with 0.1 mm for the controls. And increasing the strength of vaccine caused a gradual increase in the level of allergy, about 2 mm in average reaction size for each tenfold increase in dose, up to an average of 10.9 mm for the strongest (10 st.) living BCG. The most concentrated vaccine used in the project, 100 st. killed BCG, gave reactions averaging only 5.4 mm—significantly greater than in the controls but less than obtained with 1/100 st. living vaccine, which is only 1/10,000 as concentrated as the killed vaccine.

The mean size of the tuberculin reactions may also be used to characterize the group's response to a given vaccine. Fig. 5 shows a linear increase in the degree of allergy with the logarithm of the dose. The two lines (and the two points) represent the findings for the two different sites on the abdomen. The lines are nearly parallel, but the reactions in the lower quadrant were consistently 2-3 mm larger than those in the upper. There is no indication that a maximal level of allergy has been approached by the strongest dose of living BCG used : the lines show no tendency to level off with increasing dose. Still larger doses of living vaccine might therefore be expected to produce still higher degrees of allergy. On each site, the reactions produced by dead BCG had a smaller mean size than those produced by 1/100 st. living BCG.

FIG. 5. MEAN SIZE OF TUBERCULIN REACTIONS ON TWO DIFFERENT SITES, 32 DAYS AFTER VACCINATION, FOR GROUPS OF GUINEA-PIGS, ACCORDING TO STRENGTH OF BCG VACCINE

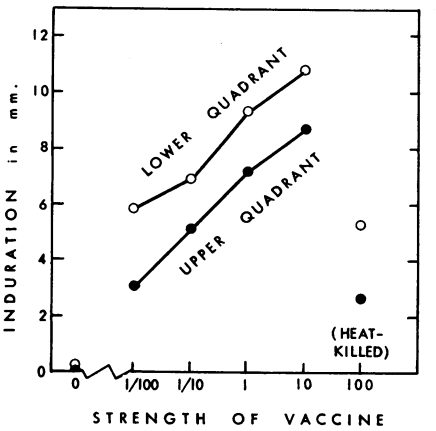


FIG. 6. PERCENTAGE OF GUINEA-PIGS STILL ALIVE AT DIFFERENT TIMES AFTER INFECTION WITH VIRULENT TUBERCLE BACILLI, FOR GROUPS OF GUINEA-PIGS VACCINATED WITH DIFFERENT STRENGTHS OF BCG VACCINE

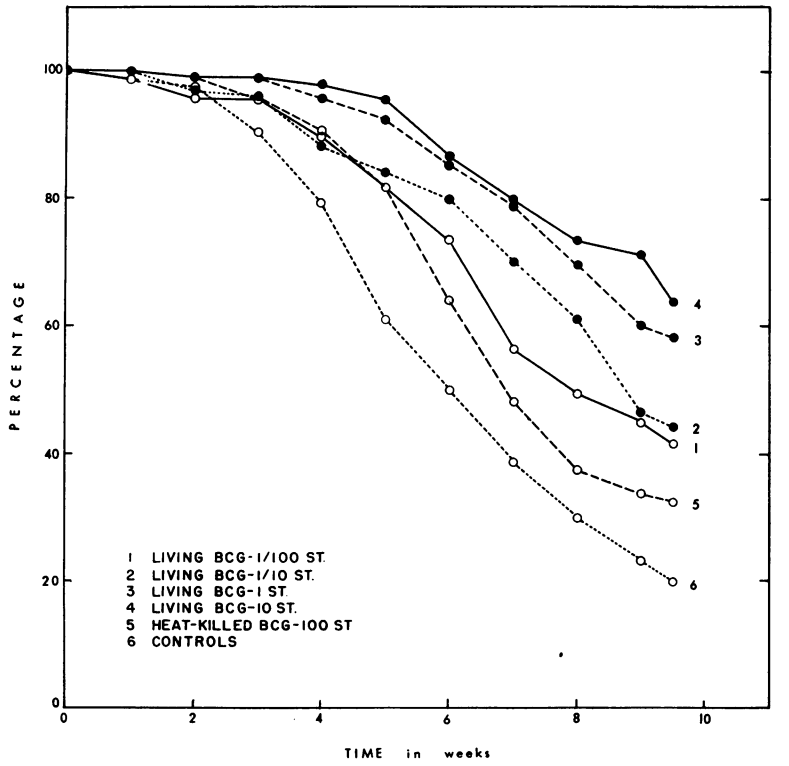


Fig. 6 shows the length of time the animals vaccinated with the different vaccines survived after challenge infection with virulent tubercle bacilli. Survival time in weeks is plotted on the abscissa, and a curve for each group indicates the percentage of animals still alive at different intervals up to 9½ weeks (when the experiment had to be stopped prematurely). There were no conspicuous differences between the six groups during the first two weeks, but by the third week the control group had already dropped below the others. By the sixth week, as is also shown in fig. 7, all groups were arranged in an order they kept throughout the rest of the observation period. Animals vaccinated with the strongest concentration of living BCG lived longest; the other groups followed according to the size of the dose. In contrast, the killed BCG, although used in 100 times the standard strength, had less effect than the weakest dose of living BCG.

FIG. 7. PERCENTAGE OF GUINEA-PIGS STILL ALIVE AT 6 WEEKS AND 9 ½ WEEKS AFTER INFECTION WITH VIRULENT TUBERCLE BACILLI, FOR GROUPS OF GUINEA-PIGS, ACCORDING TO STRENGTH OF BCG VACCINE

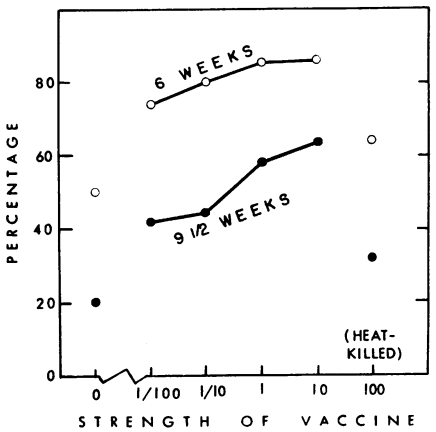


Fig. 8 correlates the degree of post-vaccination allergy with the chances of surviving 8 weeks after challenge infection. Each point, labelled with the kind and strength of vaccine, shows the mean size of the tuberculin reactions (abscissa) and the survival percentages (ordinate) for each experimental group. The four points representing the four dilutions of living vaccine have been connected by a line to show how, as dose is reduced, there is a corresponding drop (as might be expected) in both degree of allergy and survival time. The point for the heat-killed vaccine falls below, but not far from, the curve that might be drawn as a continuation of the one representing the various doses of living vaccine. This result suggests that the relation between allergy and survival is not conspicuously changed when killed BCG is used for vaccination.

FIG. 8.
RELATION BETWEEN PERCENTAGE OF GUINEA-PIGS STILL ALIVE 8 WEEKS AFTER INFECTION WITH VIRULENT TUBERCLE BACILLI AND MEAN SIZE OF POST-VACCINATION TUBERCULIN REACTIONS, FOR GROUPS OF GUINEA-PIGS VACCINATED WITH DIFFERENT STRENGTHS OF BCG VACCINE

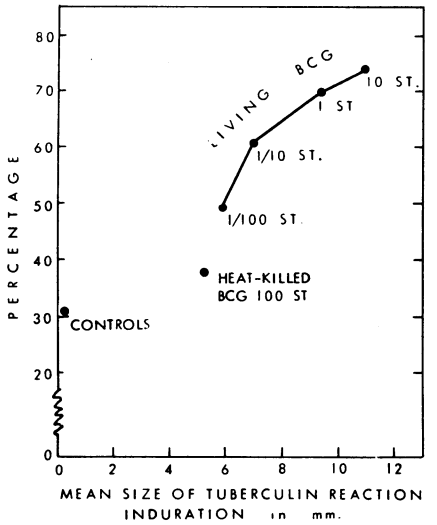
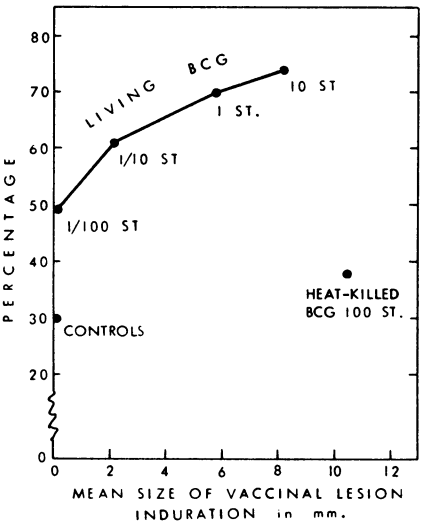


FIG. 9.
RELATION BETWEEN PERCENTAGE OF GUINEA-PIGS STILL ALIVE 8 WEEKS AFTER INFECTION WITH VIRULENT TUBERCLE BACILLI AND MEAN SIZE OF VACCINAL LESIONS, FOR GROUPS OF GUINEA-PIGS VACCINATED WITH 4 DIFFERENT STRENGTHS OF BCG VACCINE



In correlating mean size of vaccinal lesion (at 10 days) with survival time in a corresponding way, as shown in fig. 9, the same close association is found between the two effects as long as living vaccine is used in different doses. The smaller the lesion, the shorter the survival time; and, as indicated by the group vaccinated with the 1/100 strength vaccine, the lesion may become imperceptible though the survival time is still measurably longer than in the controls. A pronounced dissociation occurs between lesion size and survival time when the effects of heat-killed and living vaccines are compared : the point for the group vaccinated with killed BCG lies far below the line that describes the relation for different doses of living vaccine. Ability to survive has apparently been affected to a much greater degree than might be expected from the size of the vaccinal lesion.

Discussion

This first report of results of the research programme deals with one segment of the complex allergy-immunity problem : the effect of varying the dose of BCG on response to vaccination. Variations in dose have previously been studied extensively in human beings where the effect of

vaccination is necessarily limited to two measurable responses : tuberculin allergy and vaccinal lesions. The present project on guinea-pigs was designed to repeat, as nearly as possible, the studies on human beings in order to compare the dose-response relation in man with that in guinea-pigs.

Post-vaccination tuberculin allergy in guinea-pigs, as in human beings, is appropriately described in terms of degree; and the average size of the tuberculin reactions of a vaccinated group gives a fair expression of the degree of allergy produced by a particular sample of vaccine. There is considerable variation among the individuals within a group, yet when the group contains a sufficient number of subjects, humans or guinea-pigs, the tuberculin reactions are usually distributed, by size, to form a unimodal frequency curve. And the effect of varying the dose of BCG vaccine is to vary the degree of post-vaccination allergy, each tenfold decrease in dose causing a small but significant decrease in mean reaction size.

Varying the dose of BCG vaccine thus appears to have about the same effect on post-vaccination allergy in guinea-pigs as in human beings, but the general level of allergy differs considerably in the two species; as is well known, guinea-pigs are much less sensitive to tuberculin than man. Given the same dose of vaccine, the animals require a dose of tuberculin about 100 times stronger than that used in humans to bring out reactions measuring about the same size. In addition to this lower level of sensitivity, guinea-pigs also show a smaller change in mean reaction size with change in dose of BCG; though the tuberculin reaction in both species has a linear relation to the logarithm of the dose, the guinea-pig is somewhat less sensitive than man to variations in dose.

Guinea-pigs and human beings are even more alike with respect to the vaccinal lesion than they are with respect to allergy. The same dose of vaccine in the two species produces lesions of about the same average size, and varying the dose causes corresponding variations in the lesions. Development of the lesion probably depends on local tissue response, and in this respect guinea-pigs apparently are no less sensitive than man to vaccine injected into the skin.

The similarities between the two species in pattern of response to variations in the dose of vaccine holds, also, for changing the composition of vaccine from living to dead BCG organisms, at least as far as allergy and lesions are concerned. As compared with living vaccine, dead BCG causes a very low degree of allergy, but the size of the vaccinal lesion is apparently unaffected. This dissociation between effects with dead as compared with living BCG is of immediate practical significance, for it has been commonly accepted that the " potency " of a vaccine may be estimated from the size of the skin lesion in guinea-pigs. The present results, though preliminary, would seem to indicate that while the lesion may reflect the dose of vaccine injected, it is of dubious value as an index of the vaccine's immunizing potency.

Resistance to virulent infection as measured by survival time in guinea-pigs is, like allergy, a matter of degree. And, like allergy, the resistance of groups of animals is directly related to the dose of BCG vaccine given : increasing the dose of vaccine increases both the degree of allergy and the degree of resistance.[b]

The results of the present study, expressed in quantitative terms, are compatible with the view of many experimental workers that allergy and immunity are closely associated (Bindslev,[3] Birkhaug,[4] Mande,[16] Nègre & Bretey,[18] Yanagisawa [23]). The association between the sensitizing and the immunizing properties of a vaccine is, however, no proof that hypersensitivity plays any part in immunity to tuberculosis; but it is not our intention here to discuss that oft-debated subject (reviewed in extenso by Rich [21]). Nevertheless, the present results certainly indicate that, among BCG vaccines containing various concentrations of living organisms, the best immunity in guinea-pigs (longest survival time) is produced by the vaccines that also produce the strongest degrees of tuberculin allergy, and that, in contrast to the vaccinal lesion, the tuberculin reaction measured a few weeks after vaccination appears to be a useful index of the resulting immunity.

The results would also seem to have some implications for current BCG vaccination programmes in man. Clearly, the material is much too restricted both in extent and in substance to permit more than speculation. Yet, and without elaborating on the obvious limitations, it would seem worth while to mention a few points.

First, and certainly the most pertinent, is the implication that the higher the degree of allergy a particular vaccine produces, the better the protection. This would tend to support those who advocate the desirability of producing strong post-vaccination tuberculin reactions (Groth-Petersen,[12] International Tuberculosis Campaign,[14] Ranganathan [20]) rather than those who believe that allergy is unnecessary or undesirable after vaccination (Assis,[2] Gines & Gould,[10] Holland,[13] Sayé [22]). Moreover, it offers little support to those who seek a more potent tuberculin or use a larger dose of tuberculin to " bring out " weak post-vaccination allergy (Aronson & McGettigan,[1] Delachaux & Bergier,[5] Epifano,[8] Pangborn & Birkhaug [19]). Second is the implication that the size of the vaccinal lesion, of the " take " at the site of vaccination, is not a reliable sign of the potency of the vaccine, either in the BCG production centre or in the vaccination of human beings. And, thirdly, the results would encourage those who want to experiment with stronger doses of BCG than the ones currently in use, but have hesitated for fear of too large vaccinal lesions. Moreover, if further research confirms the need for strong post-vaccination allergy, we may then be obliged to find the upper limits of BCG dosage that can be tolerated in large-scale intradermal vaccination programmes.

[b] The critical problem of the correlation between allergy and immunity in the *individual* animal will be considered in later papers.

RÉSUMÉ

Cet article expose les résultats d'une expérience faisant partie d'un vaste programme de recherches de laboratoire grâce auquel on se propose d'étudier la relation entre les réactions post-vaccinales observables et la résistance acquise à la tuberculose. Il a été antérieurement constaté qu'en faisant varier la dose de BCG administrée, on obtient chez l'homme des variations systématiques dans le degré de sensibilité cutanée à la tuberculine et dans la dimension des lésions vaccinales : l'expérience analysée ici avait pour objet d'étudier si des variations analogues se produisent chez le cobaye, puis, en infectant les animaux au moyen de bacilles tuberculeux virulents, d'examiner la corrélation éventuelle entre la durée de survie d'une part, l'allergie tuberculinique et les lésions vaccinales d'autre part.

Cinq groupes de cobayes, comprenant chacun 120 animaux, ont été vaccinés avec quatre différentes doses d'un vaccin BCG récemment préparé, comprises entre 1/100 et 10 fois la dose ordinairement utilisée pour la vaccination humaine par voie intradermique, et avec un vaccin BCG tué par la chaleur, à une dose égale à 100 fois la dose normale. Un sixième groupe de 120 animaux n'a pas été vacciné. La tuberculino-réaction a été pratiquée sur tous les animaux immédiatement avant la vaccination, puis cinq semaines après; une forte dose d'épreuve de H-37 Rv a ensuite été administrée à tous ces animaux, que l'on a laissé mourir spontanément afin d'évaluer la résistance, exprimée par la durée de survie.

Les résultats obtenus sur ces différents groupes de cobayes montrent que plus la dose de BCG vivant est forte, plus le degré moyen d'allergie tuberculinique post-vaccinale augmente, de même que le diamètre de la lésion vaccinale et la durée de survie après infection par des bacilles virulents. Le BCG tué par la chaleur provoque chez les animaux une allergie nette mais très faible; la durée de survie est corrélativement plus courte, bien que les lésions vaccinales soient en moyenne aussi grandes qu'elles l'auraient sans doute été si l'on avait administré la même dose de BCG vivant. Ces résultats correspondent exactement à ceux que l'on a enregistrés chez les êtres humains (exception faite des résultats relatifs à la durée de survie).

Les conséquences que ces résultats pourront avoir pour la pratique des vaccinations en série par le BCG sont encore d'ordre spéculatif. Toutefois, les expériences faites semblent indiquer qu'il serait peut-être avantageux de provoquer une forte allergie tuberculinique; ils montrent aussi que la lésion vaccinale n'a qu'une valeur fort douteuse pour mesurer le pouvoir immunisant d'un vaccin.

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