THE CORRELATION OF A BIPHASIC METABOLIC RESPONSE WITH A BIPHASIC RESPONSE IN RESISTANCE TO TUBERCULOSIS IN RABBITS*

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Plates 76 to 79

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We have demonstrated that peritoneal mononuclear exudate cells from normal natively resistant rabbits dehydrogenated certain substrates to a greater degree than similar cells from natively susceptible animals (1). We then showed that BCG vaccination raises the level of utilization of certain but not necessarily the same substrates by these mononuclear cells above that of cells from rabbits which had not received BCG (2). Before this period of heightened substrate utilization, we noted a period of depressed utilization with many substrates in BCG-vaccinated animals. Thus, both native high resistance and the increased acquired resistance following BCG vaccination were associated with a higher level of certain metabolic activities of the mononuclear phagocyte than these found in the susceptible animals or in the nonvaccinated animals. In our present experiment we are interested in determining if there is any relationship between resistance to tuberculosis and the depressed period previously mentioned in BCG vaccination, for if this is so, then we would have a biphasic response in resistance that would correlate with our biphasic response in metabolism.

Materials and Methods

Rabbits.—The race of rabbits chosen for this work was started in 1945 by crossing Lurie's original resistant race A with his intermediate race D. This AD race originally was of intermediate resistance and required the inhalation of about 360 human tubercle bacilli for each tubercle formed in the lungs. By the eighth generation, this race had lost its original resistance and resembled the more susceptible races. At this time, less than 30 inhaled human tubercle bacilli were required to generate one tubercle in the lungs.

Method of BCG Vaccination.—All animals that received BCG vaccination were given 1 mg

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intradermally of Phipp's Strain 873 suspended in Sauton's buffer solution. This was followed six weeks later by a second injection of one mg of the same BCG, subcutaneously. The viable count for this strain averages 30 million organisms per milligram.

Method of Infection.—BCG-vaccinated and control animals were infected 4 days and 28 days after the subcutaneous injection of BCG. All animals, both control and experimental, were infected by quantitative airborne infection with a suspension made from a 6 day growth of H37 Rv human tubercle bacilli from a modified Lowenstein medium (3). The method of infection is described in detail in previous publications as well as its use for the quantitative evaluation of resistance (4-6). In brief, it involves the calculation of the number of tubercle bacilli inhaled and relating this number to the number of individual tubercles found in the lung 28 to 34 days after infection. Definite ratios have been established for the different races of rabbits, for example, as many as 1000 human tubercle bacilli may be needed to obtain one tubercle in the lung of a resistant rabbit, whereas as few as 30 may be needed in a very susceptible animal.

Metabolic Studies.—All animals were killed by air embolism 28 to 34 days after infection, having first received mineral oil intraperitoneally four days before death. The exudates were collected, standardized, and tested as previously described (1). The lungs were saved for tubercle counts and complete autopsies were done on each animal. Sections of lung were taken and stained with hematoxylin and eosin as well as with Ziehl Neelson method for tubercle bacilli.

OBSERVATIONS

The Effect of Reinfection on Substrate Utilization by BCG-Vaccinated Rabbits.—Table I gives the values for substrate utilization by peritoneal mononuclear phagocytes from tuberculous non-vaccinated, tuberculous BCG-vaccinated, and completely normal race AD rabbits. The BCG-vaccinated animals were further subdivided into those infected 4 days and those infected 28 days after the subcutaneous injection of BCG. Our observations at the 28 day level are limited to two observations due to an error in choosing the animals, but these two observations are so characteristic that we feel they should be mentioned.

The cells from rabbits exposed to infection 4 days after vaccination and tested some 28 days after challenge had a higher utilization of certain substrates than cells from tuberculous controls. These substrates were succinate, glycerophosphate, beta hydroxybutyrate and glycerol. Although these substrates were utilized more by the vaccinated animals' cells than by those of the controls, the other five substrates tested showed no differences in usage.

Comparing those animals infected 28 days after vaccination with the controls, we find that the cells from these rabbits used four to five times the amount of substrate in a given time interval, with all substrates studied.

The protein nitrogen in all groups was essentially the same as normal: 1.16 $\pm$ 0.07 per 10 million peritoneal exudate cells.

The tuberculous non-vaccinated rabbits generally had a lower total cell count (66×10^6) than normal for this race (136×10^6). Those infected four days after vaccination had about the same total cells as normal tuberculous non-vaccinated rabbits; those infected 28 days after vaccination had higher

than normal responses (182×10^6). This is the typical heightened response of the immune animal. The differential count of the exudate cells was essentially the same in all groups.

The Relation of Metabolic Response in Resistance to Tuberculosis.—Table II gives the ratios for the number of tubercle bacilli required to generate one

TABLE I

The Effect of Virulent Reinfection on the Metabolic Activity of Peritoneal Mononuclear Exudate Cells from BCG-Vaccinated, Race AD Rabbits

Values are for 10 million cells.

Substrate	Normal controls	Tuberculous controls	BCG-vaccinated infected 4 days later	BCG-vaccinated infected 28 days later
Lactic acid	0.032 ± 0.007 (9)*	0.019 ± 0.004 (5)	0.029 ± 0.006 (4)	0.084 (2)
Glucose 6 PO_4	0.030 ± 0.004 (10)	0.022 ± 0.003 (3)	—	0.108 (2)
Sodium succinate	0.024 ± 0.005 (9)	0.017 ± 0.003 (9)	0.042 ± 0.011 (4)	0.106 (2)
Malic acid	0.013 ± 0.002 (9)	0.016 ± 0.002 (7)	0.021 ± 0.006 (4)	0.097 (2)
Sodium glycero PO_4	0.040 ± 0.004 (10)	0.028 ± 0.005 (9)	0.064 ± 0.016 (4)	0.148 (2)
Beta-OH butyrate	0.027 ± 0.005 (9)	0.017 ± 0.003 (8)	0.040 ± 0.009 (4)	0.113 (2)
Glycerol	0.022 ± 0.004 (9)	0.010 ± 0.004 (7)	0.036 ± 0.006 (4)	0.098 (2)
Isocitric acid	0.026 ± 0.005 (9)	0.022 ± 0.003 (3)	—	0.122 (2)
Triose PO_4	0.025 ± 0.005 (9)	—	—	—
Alpha keto-glutarate	0.017 ± 0.002 (9)	—	—	—
Glutamic acid	0.014 ± 0.004 (8)	—	—	—
Acid phosphatase‡	1.73 ± 0.21 (8)	1.86 ± 0.33 (6)	1.87 ± 0.33 (4)	2.49 (2)

* Numbers in parentheses indicate No. of observations.

‡ mgm per cent P/hr.

All other values are slope of curve for reduction of methylene blue.

tubercle in the lungs of control, and BCG-vaccinated rabbits infected 4 and 28 days after the subcutaneous BCG vaccination. In addition, the average size of the tubercle is also included.

In those animals infected 4 days after vaccination, we found a considerably lower ratio of tubercle bacilli per tubercle than in the controls, indicative of a greater susceptibility, with the formation of many more tubercles. Since the rabbits were killed over a 6 day period, we observed that those vaccinated rabbits killed at 28 days after infection had larger tubercles than the same vaccinated rabbits killed 34 days after infection. On the other hand, the controls showed an increase in tubercle size for this same interval. These observa-

TABLE II

Ratios of the Number of Tubercle Bacilli Needed to Yield 1 Tubercle Lung after the Quantitative Inhalation of Human tubercle bacilli

Rabbit	Ratio Tubercle bacilli/Tubercle	Tubercle size
		mm
Control, non-vaccinated rabbits		
AD-752	24.0	3.5
AD8-44	13.3	3.5
AD8-41	12.2	4.0
AD8-42	14.0	4.0
AD8-38	22.2	3.6
AD8-43	16.1	4.3
AD8-46	27.9	5.2
Average	18.5 ± 2.3	4.0
BCG-vaccinated rabbits infected during period of metabolic depression		
AD7-49	7.4	3.5
AD7-50	13.2	3.0
AD7-53	8.5	3.0
AD8-47	10.1	3.0
AD8-48	9.3	2.0
AD8-49	8.5	2.5
AD9-3	4.3	1.5
Average	8.7 ± 1.3	2.6
BCG-vaccinated rabbits infected during heightened metabolic activity		
AD8-32	16.4	3.2
AD8-34	24.0	2.5
AD8-35	53.0	1.5
AD8-36	32.7	2.6
AD8-37	42.8	2.0
Average	33.7 ± 6.6	2.3
BCG-vaccinated rabbits, irradiated 400 r 2 years before		
AD7-55	25.1	2.7
AD7-59	5.9	2.3
Average	15.5	2.5

tions are clearly brought out in Figs. 1 to 4. Histologically, the vaccinated animals had smaller tubercles with less caseation and containing fewer tubercle bacilli than the controls. Thus, we have clear experimental evidence of heightened susceptibility to initiation of this infection but an increased resistance to the progression of the same infection, in the vaccinated animals. This increased susceptibility was correlated with an initially depressed metabolic activity at the time of infection, as was shown for races III and CAC^2, which nevertheless rose somewhat in some cases to a higher level than that of the controls at the time of death 28 to 34 days later. See Fig. 2B for a typical section.

Those rabbits infected 28 days after the subcutaneous vaccination showed a heightened resistance to attack by tubercle bacilli. The ratios for these vaccinated animals was higher than the controls, that is, it required more bacilli to generate one tubercle. This is illustrated in Figs. 5 and 6. Histological study shows the usual resistance to progression of disease as manifested by small tubercles, little caseation and few tubercle bacilli. See Figs. 5B and 6B.

The Effects of Irradiation on Resistance to Tuberculosis.—Two rabbits that had been given 400 roentgen units two years previously had been treated with BCG and included in the immune group infected 28 days after vaccination. These animals showed no response to BCG vaccination, as the ratio of tubercle bacilli needed to generate one tubercle was comparable to the controls. The metabolic activity of these rabbits macrophages were also on a low level, comparable to the controls. The total number of cells recovered from the peritoneal cavity was low, again comparable to the controls. The lungs of one of these rabbits is illustrated in Fig. 7. Histologically, these animals did have smaller tubercles than the control, but these lesions had numerous tubercle bacilli within them as seen in Fig. 7B.

DISCUSSION

We have demonstrated a biphasic type of resistance to tuberculosis after BCG vaccination that coordinates with a biphasic reaction in the utilization of metabolic substrates. A period of depressed resistance occurred on the 4th day after subcutaneous injection of BCG. This period is one in which the utilization of many different substrates by peritoneal mononuclear exudate cells is also depressed. 28 days after the vaccination with BCG, the animals had a period of increased resistance that was associated with a period of heightened metabolic activity on the part of the mononuclear exudate cells. The alteration in resistance was primarily in that phase of resistance that involved the initiation of the infection. It would appear that the vaccinated animals even when infected at depressed metabolic levels, had the ability to recover rapidly and respond more effectively to the infection than the controls.

Thus, we have seen here experimental evidence of the old German clinical observation of *anfälligkeit* and *hinfälligkeit*; we find that the BCG-vaccinated animals from

the four day period have more tubercles but that they are smaller than the controls, show less caseation, and contain fewer tubercle bacilli. In a previous work, we demonstrated that the natively resistant rabbit was able to inactivate or destroy about 50 per cent of either human (7) or bovine (6) tubercle bacilli inhaled, within the first three days after infection. The susceptible animal was unable to do this, hence we found more tubercles in the susceptible animal than we did in the resistant animal. Lurie found that the natively resistant animal responded to infection much as a vaccinated animal of a less resistant race. We must then conclude in our present experiment that the mechanism involved in this initial reaction to infection, in those animals infected at the 4 day interval, was either absent or working at a very low level so as to be ineffective. However, the mechanism has the ability to recover rapidly, unless some alternate pathway is involved, for the progression of the tubercles is inhibited. The answer to this must lie somewhere in the metabolic scheme, for the BCG-vaccinated rabbit infected four days after vaccination presumably started at a considerably lower level of metabolic activity, and yet at the time of death about 30 days later in a number of reactions these rabbits used more substrate than the unvaccinated tuberculous controls.

Krause and Willis in 1920 published a study that is related to our findings. They found that guinea pigs infected initially with the strain R1 of the tubercle bacillus when injected with W.E. (a watery extract of desiccated, pulverized human tubercle bacilli used as tuberculin) and then reinfected in different locations with virulent H37 human tubercle bacilli, had a reduction in immunity and allergy. This occurred within one to four days after the application of tuberculin and was greater on the side of the tuberculin reaction. Their measurement of immunity was the extent of the disease in the organs graded from one to four plus. Although the controls showed more extensive disease than any of the vaccinated guinea pigs, there was a considerable loss of resistance in the vaccinated animals, particularly one to two days after administration of W.E. While the measurement of resistance that these authors used was not a really quantitative one that would enable them to differentiate between resistance to infection and resistance to progression of the disease, nevertheless they do show a similar period of depression in resistance as we have had in another species.

Biphasic responses in resistance have been observed in a number of different conditions and with a number of different disease entities. During the course of non-specific protein therapy that was in vogue just prior to and after the First World War, it was shown that there was a phase of depressed resistance that occurred prior to the increase in resistance following this non-specific protein therapy. When we speak of non-specific protein therapy, we are covering a number of stimulatory agents other than protein, such as counterirritants, colloidal metals, and various protein split products, as well as proteins of animal and plant origin. It was only natural that a similar biphasic reaction in resistance should occur under certain conditions with the use of endotoxins (10, 11) since the bacteria from which these endotoxins were obtained were often used in non-specific protein therapy. It is very possible that these endotoxins in many cases were actually the active factors responsible for the changes that occurred in the patient when non-specific protein therapy was used, for the presence of pyrogens was not considered in the use of these non-specific substances. The general non-specific aspects of BCG vaccination which are reflected in the altered resistance

to heterologous infections are by now well known (11–13). Our present work that demonstrates an altered resistance associated with altered metabolism adds to the evidence that is accumulating to place BCG among the agents inducing non-specific resistance, and that resistance to tuberculosis is at least in part in the category of non-specific (non-antibody) resistance.

A discussion of the changes that are responsible for this altered resistance must always bring us to a discussion of metabolic factors. A number of years ago, Weichardt (14) in explaining the mechanism of non-specific protein therapy, maintained that it resulted from a stimulation of the cells to a greater activity. This was reflected in a rise in cellular metabolism, speed-up of detoxification, and was a result of mobilization of enzymes and antibodies on a general, non-specific, level. In brief, the infection and intoxication are overcome not by new means of defense, but by the accentuation of mechanisms already present in the cells. The mechanisms of action of endotoxin are not very clearly defined, but certainly they are similar to some of those ascribed to non-specific protein therapy. The effects of endotoxin are certainly of a general nature involving many different tissues and cells, irrespective of the portal of entry. Cohn and Morse (15) concluded that endotoxin could alter the metabolic properties of polymorphonuclear leukocytes increasing the utilization of glucose and production of lactic acid. Howard (16) demonstrated an increase in acid phosphatase in the Kupffer cells of mice who had been given endotoxin. The work of Whitby and Rowley (17) suggests that endotoxin stimulates the production of specific and/or "nonspecific" opsonic materials which enhance the efficiency of phagocytosis. Finally, increased antibody production has also been noted in endotoxin-treated animals by Johnson, Gaines, and Landy (18). BCG vaccination apparently has a mechanism of action very similar to non-specific protein therapy and endotoxin. Our own work (2) has demonstrated a different metabolic state from normal, increased capacity for antibody production (19) has been demonstrated, as well as increased phagocytic capacities (13) which in turn have been shown to depend on opsonins. Changes in digestive capacities of the mononuclear phagocytes (13) have also been demonstrated. Thus we find a number of common denominators in endotoxin, non-specific protein therapy, and BCG vaccination that could reside in similar metabolic activities, and these are correlated with biphasic differences in resistance to disease.

There is one major difference in these various groups—that is the time of duration of the altered state of resistance. Since endotoxin is probably closest to the basic principle responsible for these changes, it is probably the one with the shortest duration, closely followed by non-specific protein therapy. BCG vaccination gives a more prolonged response possibly due on the one hand to the nature of the bacillus, which is very resistant to digestion and on the other, due to its capacity to multiply, albeit slightly, in the tissue. Hence, the active principle is probably released slowly and over a longer period of time. A basis for this is suggested by the study of growth curves of the tubercle bacillus in rabbits and the simultaneous study of histological tissues in the same animal.

In conclusion, we must state that a biphasic stage has been noted in resistance to tuberculosis in rabbits that is related both to the initiation of the disease

and closely associated with a biphasic metabolic response on the part of the mononuclear cell.

SUMMARY

We have found a phase of susceptibility associated with a reduced metabolic activity on the part of peritoneal mononuclear phagocytes taken from BCG-vaccinated rabbits. A second stage of heightened resistance to infection was found to be associated with a heightened metabolic activity. The period of susceptibility in BCG vaccination is primarily concerned with initiation of the infection and not with the progression of the disease, which in both stages is increased.

These reactions are discussed in relation to other conditions, such as non-specific protein therapy and the administration of endotoxin, which also have similiar biphasic stages of resistance.

Of incidental interest is the fact that rabbits who received 400 roentgen units are two years later still unable to respond to BCG vaccination with an increase in resistance.

We conclude that there is a relationship between the level of certain metabolic activities of reticuloendothelial cells and resistance to tuberculosis.

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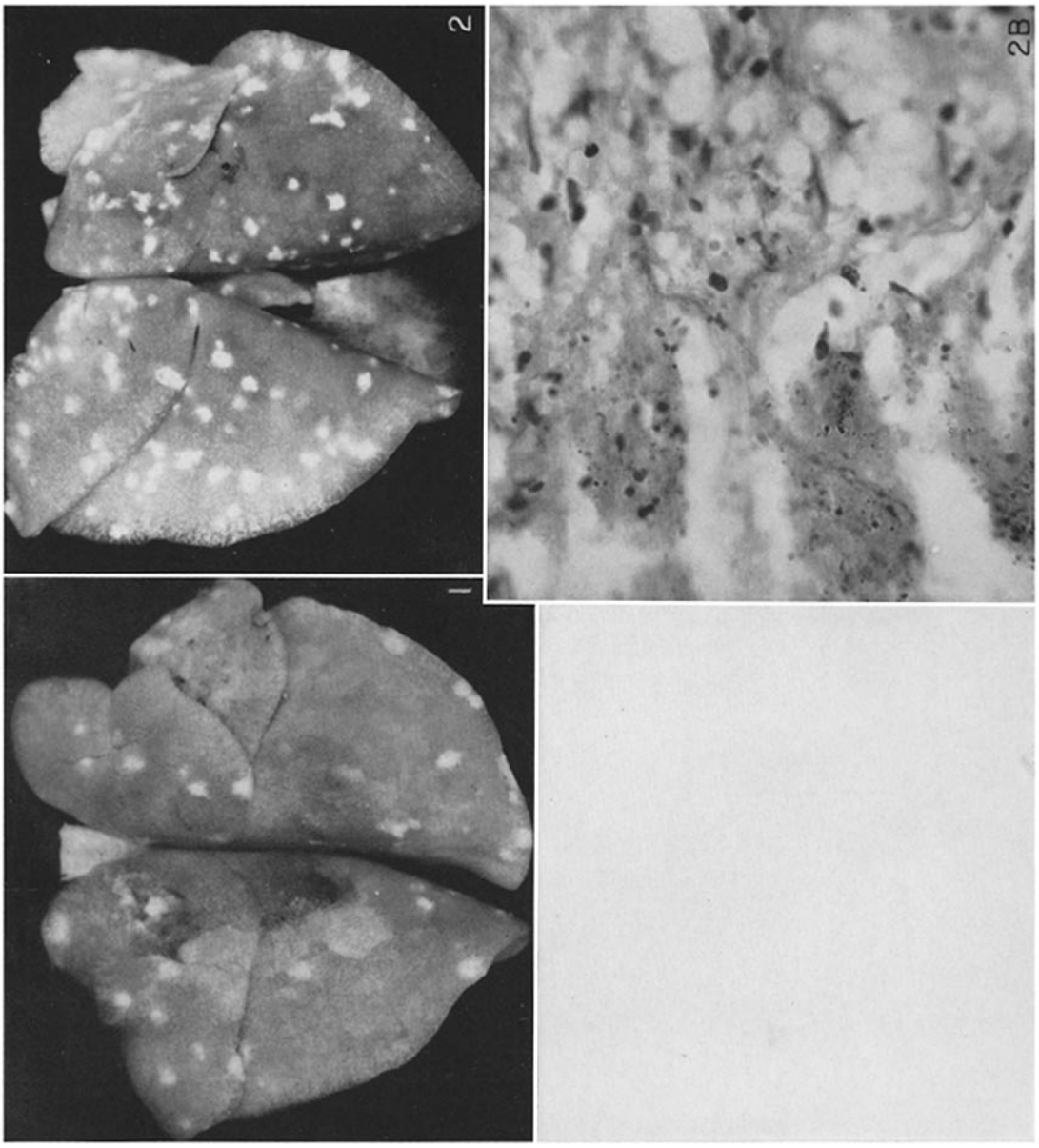
EXPLANATION OF PLATES

PLATE 76

FIG. 1. This control rabbit AD7-52 was killed 28 days after infection. It had a ratio of 24 human tubercle bacilli to each tubercle produced in the lungs. These tubercles averaged 3.5 mm in size.

FIG. 2. BCG-vaccinated rabbit AD7-49, infected 4 days after the second injection of BCG and killed simultaneously with the control in Fig. 1, had a ratio of 7 human tubercle bacilli to each tubercle. The average tubercle size was 3.5 mm. This animal is obviously more susceptible to initiation of the disease than the above control.

FIG. 2B. The microphotograph shows the few tubercle bacilli that are found in the lung of the animals represented in FIG. 2. These animals are more resistant to the progression of the disease than the controls, in spite of the more numerous tubercles and lower tubercle bacilli/tubercle ratio. × 900.

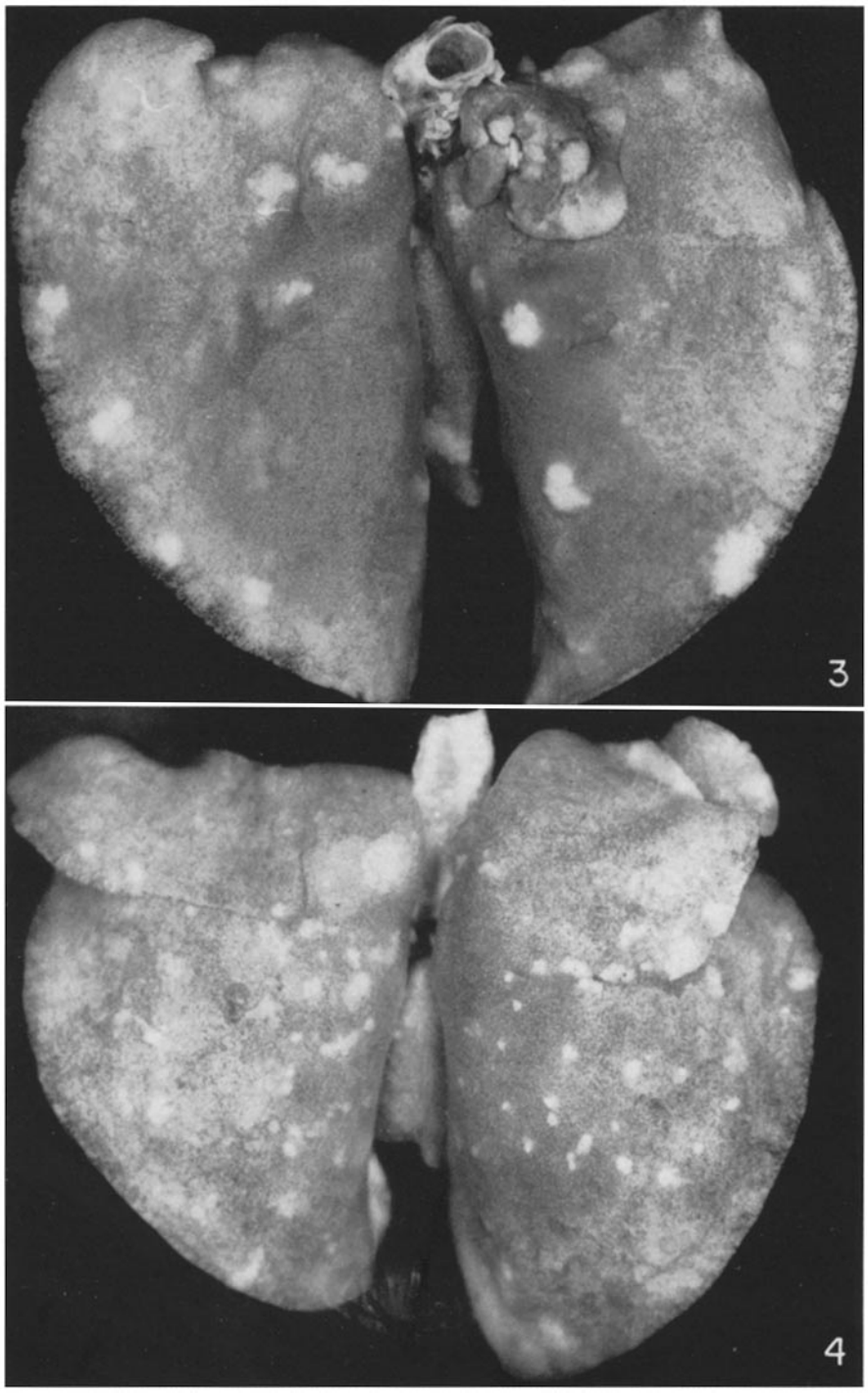


(Allison *et al.*: Resistance to tuberculosis)

PLATE 77

FIG. 3. The lungs of this control animal AD8-42 killed 6 days later than the control in FIG. 1 has slightly larger tubercles than the latter (4 mm). This control had a tubercle bacilli/tubercle ratio of 14. Clearly there is no indication of resistance to progression of the disease when compared with FIG. 1.

FIG. 4. The lungs of this vaccinated animal AD9-3, killed simultaneously with the animal of FIG. 3, has smaller tubercles (1.5) than the vaccinated animal in FIG. 2 killed 6 days before. The tubercle bacilli/tubercle ratio is 4. A comparison of this animal with the vaccinated animal in Fig. 2 illustrates the resistance in the gross to progression of the disease exhibited by the vaccinated animals, although they are found to be more susceptible to initiation of the infection than the controls at this time.



(Allison *et al.*: Resistance to tuberculosis)

PLATE 78

FIG. 5. This control animal AD8-46 was killed 30 days after infection and had a ratio of 28 tubercle bacilli to each tubercle. The average tubercle size was 5.2 mm.

FIG. 5B. This microphotograph illustrates the large numbers of tubercle bacilli found in sections from the control animals. Compare with Figs. 2B and 6B. × 600.

FIG. 6. This illustrates the effectiveness of BCG vaccination. The animal AD8-37 infected during the heightened metabolic response, found 28 days after the second injection of BCG, had a ratio of 43 tubercle bacilli to each tubercle. The average tubercle size at the time of death 28 days after infection was 2.0 mm. This animal had resistance to initiation of the infection as well as to progression of the disease.

FIG. 6B. This microphotograph shows the few tubercle bacilli commonly found in the lesions of the vaccinated animals represented by FIG. 6. Note the similarity of this figure with FIG. 2B, and yet the tubercle bacilli/tubercle ratio is six times greater in this animal. × 600.

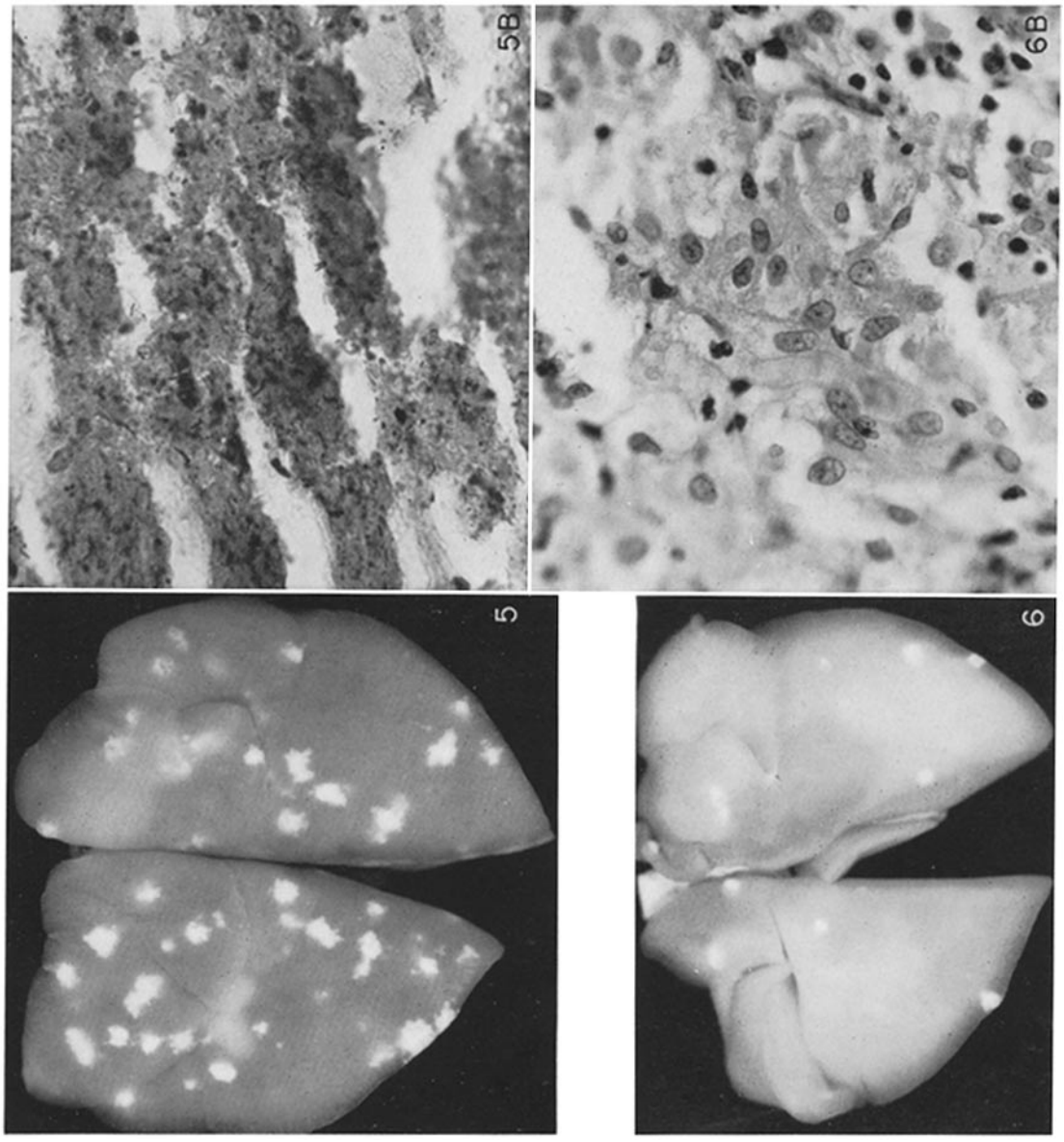


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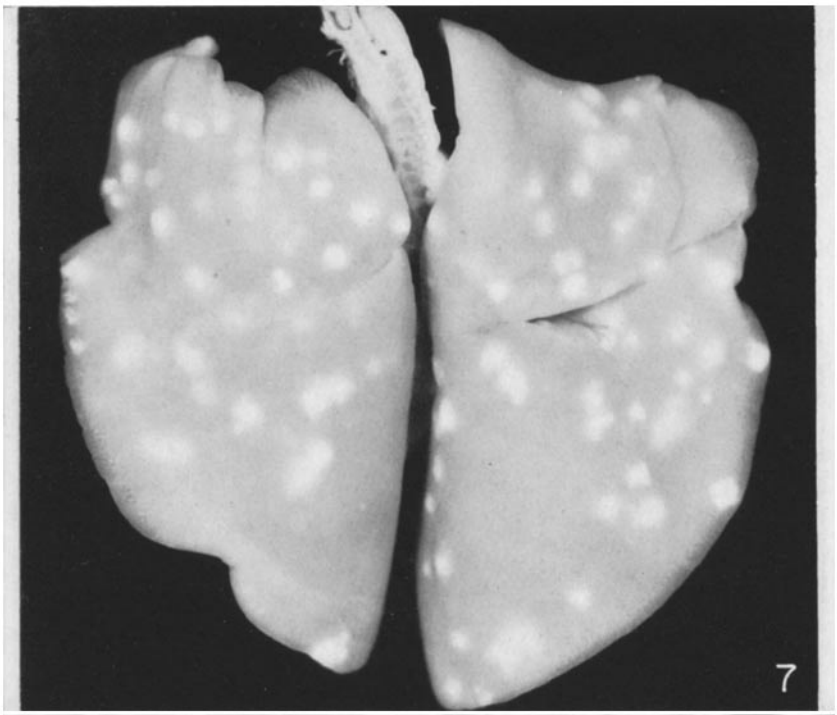
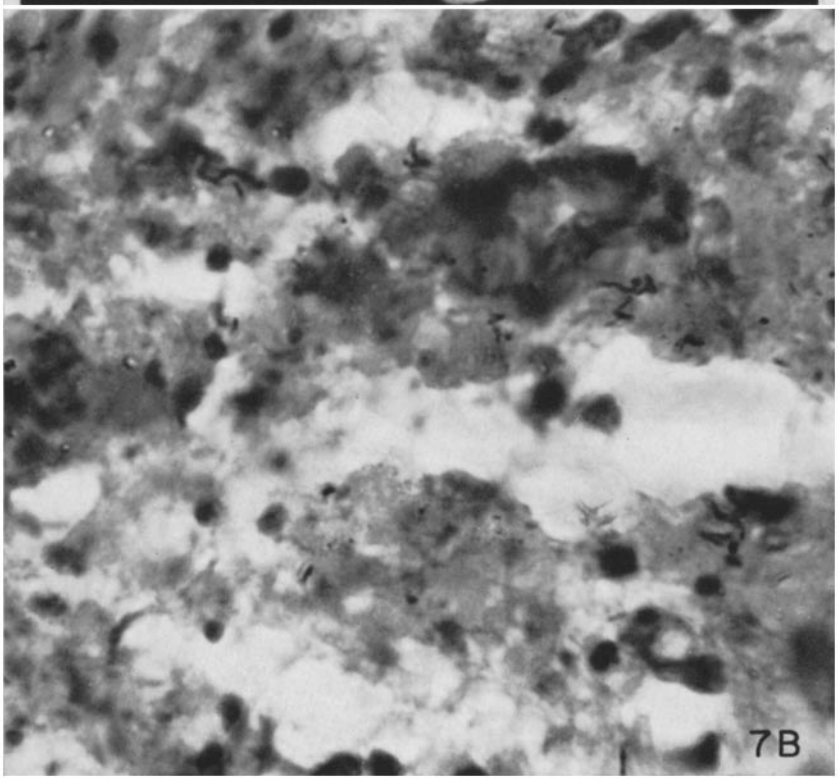
PLATE 79

FIG. 7. This rabbit AD7-55, having received total body irradiation of 400 r 2 years previously, was vaccinated with BCG and infected simultaneously with the animals of Figs. 5 and 6. The tubercle bacilli/tubercle ratio was 25, and the size of the tubercles was 2.7 mm 28 days after infection.

FIG. 7B. This microphotograph illustrates the tubercle bacilli found in the lesions from the lungs of the irradiated rabbit shown in FIG. 7. Microscopically and macroscopically, these lungs resemble Figs. 5 and 5B indicating a lack of effectiveness of BCG to raise either resistance to initiation of the disease or its progression. × 600.





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