TUBERCULOSIS INDUCED BY DROPLET NUCLEI INFECTION

Its Developmental Pattern in Hamsters in Relation to Levels of Dietary Protein *

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The development by man of effective resistance to progressive infection by tubercle bacilli is believed to be influenced to a considerable extent by levels of dietary protein and of vitamins A and C.[1-4] This opinion is based largely upon clinical and epidemiologic studies, although to some extent it finds support in observations on tuberculosis among birds at the Philadelphia Zoological Garden[5] and in experimental studies of diet and tuberculosis in mice.[6,7] However, experiments on diet and tuberculosis in rats have given conflicting results.[8,9]

An opportunity for more definitive studies of tuberculosis and nutrition has been provided by Wells's development of apparatus and techniques for quantitative study of droplet nuclei infection.[10-12] This apparatus and these techniques allow precise control of the number of tubercle bacilli that will be inhaled and deposited in the lungs. Thus, the initial intensity of infections may be predetermined at any desired level and held at that level through any number of experimental groups.[11,13]

Tuberculosis induced by droplet infection in rats, mice, guinea-pigs, hamsters, and rabbits follows a highly uniform developmental pattern in all host-parasite combinations for about 3 weeks. Thereafter, the rate and pattern of development of tubercles in the lungs and the rate of progress of the disease as a whole differ with the host-parasite combination. Within a given host-parasite combination, however, the reaction to infection is still highly uniform for a time before individual variations become evident.[14,15]

If dietary protein be a factor in the rate at which resistance develops or in its level of effectiveness,[16] its influence should be more easily detected in host-parasite combinations that are characterized by prolonged periods of uniform reaction. In experimental studies, these occur to the best advantage in guinea-pigs and hamsters that have been subjected to inhalation infection by small numbers of virulent bacilli

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of human origin, and in rats subjected to inhalation infection by numbers of virulent bacilli of bovine origin.[15]

The present report describes the effects of three levels of dietary protein upon the progress of tuberculosis induced in Syrian hamsters by droplet nuclei infection. Simultaneous experiments with rats and guinea-pigs will be presented later. These reports will amplify the summaries of certain experiments that have been published earlier.[17]

Materials and Methods

Syrian hamsters were supplied by a dealer who maintains a highly vigorous breeding stock. They were delivered to the laboratory at about 4 weeks of age with each litter as a unit accompanied by its pedigree for three generations. Identities were maintained by separate housing in small, numbered cages. The colony diet was fed until all litters attained mean average weights of at least 100 gm., at about 8 weeks of age. Weights were recorded at biweekly intervals throughout the study. Each diet group contained approximately 50 animals, the exact number depending upon a convenient combination of litters. All groups were allowed at least 6 weeks for adjusting to the diets before infections were induced, and were continued on the diets until killed for study.[18]

Infections were induced by means of Wells's apparatus and techniques.[10,11,13] As insurance against possible changes in virulence of the test organism, not less than two diet groups and usually three were infected from each suspension of tubercle bacilli during a period of no more than 5 hours. Aqueous suspension has not altered virulence and viability of the organisms during this interval.[13]

As a further check on the virulence of the organism and also on the intensity of the initial infection (i.e., the number of initial tubercles), 10 to 12 hamsters of each diet group were killed 75 to 80 days after infection. The mean number of initial tubercles in these samples of all diet groups was found to be less than 10, and in 15 of the 18 groups, less than 5. These values corresponded closely to the number of bacilli calculated to have been inhaled, again demonstrating that a high percentage of all bacilli inhaled were deposited in the lungs and induced tubercles.

Cultures of the H37Rv strain were supplied by the Standard Culture Depot of the National Tuberculosis Foundation, Trudeau, New York, and maintained on solid and liquid media.[11,12] The aqueous suspensions of bacilli used to induce infection were prepared from liquid cultures after 6 to 18 days of incubation. The rate of tubercle development was not related to the age of the culture.

The first culture was received on December 3, 1948. During November, 1952, its characteristics of growth changed abruptly. Since this change could have been associated with a loss of virulence, the culture was replaced by a second one on December 26, 1952. The virulence of this corresponded completely with that of the first, as judged by the size of the initial tubercles 75 to 80 days after infection, until February, 1954, when its virulence also changed abruptly. However, reduced virulence was not associated with visible changes in its growth characteristics. Meanwhile, it had been used to induce infection in three groups of hamsters before loss of virulence was recognized. A third culture was used for the terminal experiments of this series.

After testing several other mixtures, the isocaloric diets shown in Table I were devised and found to be about equally acceptable to hamsters, guinea-pigs, and rats, and to induce similar responses in these animals.

TABLE I
Test Diets *

	A	B	C
Salt mixture no. 12[19]	4.0%	4.0%	4.0%
Vitamin mixture†	4.0	4.0	4.0
Cod liver oil (USP)	2.0	2.0	2.0
Wheat germ oil	1.0	1.0	1.0
Cottonseed oil	4.0	4.0	4.0
Choline-cerelose (1:9)	2.0	2.0	2.0
Pulverized beet pulp	12.0	12.0	12.0
Pulverized rolled oats	25.0	15.0	5.0
Cystine-casein (1:50)	25.0	15.0	5.0
Cerelose‡	10.0	34.0	47.0
Corn syrup	11.0	7.0	14.0

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† 2 gm. each of thiamine, riboflavin, and pyridoxine; 12 gm. each of calcium, pantothenate, inositol, and nicotinic acid; 1.2 gm. of folic acid; 1 gm. of menadione; 0.08 gm. each of biotin and vitamin B_{12}; cerelose to 4,000 gm.

‡ Cerelose, trade name for dextrose prepared from corn.

Corn syrup was used as part of the carbohydrate to increase the cohesiveness and reduce wastage. Estimated levels of protein were approximately: diet A, 30 per cent; diet B, 17 per cent; and diet C, 6 per cent. Diets were mixed at intervals of 7 to 10 days and stored in inert metal containers at about 0° C. Food supplies in the cages were renewed at 2-day intervals.

These mixtures were satisfactory for hamsters and rats and, when supplemented by adequate amounts of ascorbic acid, served equally well for guinea-pigs. Diets A and B allowed reproduction and supported growth and maturation of young. The average body weight of animals on diet C usually became stable at about 85 to 90 per cent of that of animals on diet A. Animals on diet B were somewhat heavier than those on diet A. However, a more detailed comparison of body weights hardly seems justified since progressive tuberculosis often was associated with rapid loss of weight in animals on diets B and C.

The animals were killed for study 75 to 80, 125 to 130, 150 to 155, 175 to 180, and 200 days after infection. They were chloroformed and

exsanguinated by cutting axillary vessels, after which the extent and characteristics of tuberculosis in the lungs, thoracic lymph nodes, and spleen were recorded. The thoracic viscera were next removed en bloc. The lungs were allowed to collapse by gravity and were then filled with Bouin's fluid through the trachea and immersed in this fixative, with other tissues that were taken for microscopic study. Tissues were embedded in paraffin and stained to demonstrate the inflammatory reaction, the bacilli, and the connective tissue. All sections were cut and stained by one technician.

RESULTS

TUBERCULOSIS INDUCED BY BACILLI OF HIGH VIRULENCE (FIGS. 1 TO 14)

Tuberculosis developed at a highly uniform rate for about 130 days in hamsters of all diet groups that had inhaled the more highly virulent bacilli. Thereafter its rate of progress was much more variable, but always most rapid in animals on diet C (6 per cent protein).

Variations in the rate of progress of the disease after 130 days were not related to sex. In fact, these variations were quite as great among litter mates of either sex as among less closely related animals on the same diet. The range of variation seemed to be greatest among animals on diet B (17 per cent protein). Indeed, tuberculosis in this diet group almost bridged the difference between the patterns of disease in animals on diet C (6 per cent protein) and diet A (30 per cent protein). But, since this study has been concerned with average patterns of tuberculosis and their relations to levels of dietary protein, attention will be given mainly to the more significant lesions of animals on diets C and A.

Macroscopic Appearances

From 75 to 130 Days after Infection (Figs. 1 and 2). Initial tubercles of all animals killed 75 to 80 days after infection were sharply defined nodules about 5 mm. in diameter. At this time, too, secondary tubercles of the thoracic lymph nodes also measured about 5 mm. in diameter in all animals. Secondary tubercles of the lungs and spleen were much smaller and were irregularly distributed among the animals. In fact, the lungs of the animals on diet A contained few secondary tubercles, whereas these lesions were numerous in the spleen. This distribution was reversed in the lungs and spleen of animals on diet C (Figs. 1 and 2).

The second sample from these diet groups (125 to 130 days after infection) found little change in the size of the initial tubercles or in

the size and distribution of the secondary lesions. This relatively quiescent stage of the disease was followed shortly by one in which progress seemed to have accelerated rapidly in animals on diet C and in many of the animals on diet B.

From 130 to 155 Days after Infection (Fig. 3). Initial tubercles of animals on diet C measured upwards of 10 mm. in diameter at 150 days. Secondary tubercles of the thoracic lymph nodes and spleen also had enlarged considerably within this interval of about 20 days. Often, too, the lungs contained numbers of minute (miliary) tubercles, as well as scattered secondary lesions that were believed to have originated much earlier.

From 155 to 200 Days after Infection (Figs. 4, 5, and 6). From 150 to 180 days after infection one or more initial tubercles in the lungs of all animals on diet C, and of many on diet B, apparently continued to expand irregularly. Within this interval initial tubercles also underwent more or less central liquefaction, with endobronchial tuberculosis becoming increasingly pronounced by 180 days. Often, too, the more advanced stages of endobronchial disease were accompanied by severe miliary tuberculosis of the lungs, liver, and spleen, and by further increase in size of the larger secondary tubercles in the lymph nodes and spleen. The more rapidly progressive forms of tuberculosis in animals on diet C and B were regularly associated with precipitous losses in body weight, and with pronounced distortion of the lobular structure of the liver by tubercles.

In occasional animals on diet C the spleen was even smaller than at 75 days, and tubercles of the thoracic lymph nodes were no larger than at this earlier stage. Still, even in these animals, the initial tubercles had undergone central liquefaction with endobronchial spread of the infection, which corresponded to the behavior of tuberculosis in vaccinated rabbits.[20]

In contrast to the pattern of progressive tuberculosis of animals on diet C (6 per cent protein), the process seen 75 days after infection in animals on diet A (30 per cent protein) seemed to have remained unchanged or, possibly, to have undergone more or less regression during the following 100 days. Thus, in about half the animals on diet A killed 180 days after infection, all lesions were smaller and not so well defined as they had been at 75 days (Fig. 4).

At 200 days after infection, tubercles of a number of animals on diet A were even smaller and less well defined than they had been in animals killed 20 days earlier. In others on this diet, however, the disease

apparently had been reactivated, as indicated by increased size of tubercles in the lungs, lymph nodes, and spleen, and by miliary tubercles in the lungs (Fig. 6).

Reactivation of the infection in animals on diet A was associated with chronic progressive glomerulosclerosis, which was recognized within 130 days after infection and progressed slowly thereafter. Thus, at 180 to 200 days the kidneys of at least half of these animals were deeply scarred and reduced in size. The more advanced stages of renal disease were accompanied by cardiac hypertrophy. Uninfected hamsters on diet A for an equal length of time did not develop disease of the kidneys.

Microscopic Appearances

From 75 to 130 Days after Infection (Figs. 7 and 8). Syrian hamsters that inhaled bacilli of the H37Rv strain developed characteristic tubercles within 28 days.[14] These were formed by monocytes that, with polymorphonuclear leukocytes in much smaller numbers, infiltrated and thickened the walls of alveoli and bronchioles and accumulated in compact masses within alveolar spaces. Thereafter the sharply outlined initial tubercles continued to expand without necrosis or other changes for about 80 days.

Secondary tubercles of the thoracic lymph nodes also were formed by monocytes that first appeared in tiny compact groups, scattered more or less at random in the nodes, about 35 days after infection.[14] Apparently these expanded slowly to fuse into larger masses by 130 days. At that time these lesions were infiltrated by small numbers of leukocytes. In the spleen, secondary lesions also arose as masses of monocytes that expanded slowly but were not often infiltrated by leukocytes until late in the disease. At first the secondaries in the liver were simply densely massed monocytes. Later these were mixed with lymphocytes and small giant cells.

Levels of dietary protein did not modify the histologic character of these lesions within 80 days, nor apparently influence the number of bacilli that might be demonstrated in them. At this stage of the disease bacilli were still found in sections as single cells scattered in the cytoplasm of monocytes.

Again at 125 to 130 days the inflammatory reaction was essentially the same in all diet groups. Now, however, alveolar walls within the central regions of initial tubercles had sometimes disappeared into the mass of monocytes. At this time, too, inflammatory cells (monocytes and polymorphonuclear leukocytes) were undergoing fragmentation within alveolar ducts and bronchioles at points within these central

zones. Leukocytes were most abundant in these foci of necrosis (Figs. 7 and 8). Peripheral to these relatively small central zones, by far the greater part of the initial tubercles still was formed as before. That is, the monocytes and polymorphonuclear leukocytes infiltrated the walls of alveoli and bronchioles and into alveolar spaces. The perivascular lymph channels also were filled by these cells, but the walls of the arteries and veins enclosed within the lesions usually were intact or, at most, had undergone slight thickening of the intima. At this time fibrosis was limited to the pleura where it adjoined tubercles in the lung parenchyma.

Between 80 and 130 days bacilli were not appreciably increased in the tubercles of animals on diet A (30 per cent protein). However, in animals on diet C (6 per cent protein) bacilli were found in much greater numbers in all lesions except those in the liver. In fact, bacilli were rarely demonstrated in the liver, even when they had become exceedingly numerous in the lungs, lymph nodes, and spleen.

From 130 to 200 Days after Infection (Figs. 9 to 14). Between 130 and 180 days after infection, tuberculosis of animals on diet C and of a majority on diet B (17 per cent protein) was characterized by increases in the number of bacilli, first in the central liquefying foci of initial tubercles, then throughout these lesions (Fig. 9). At the same time cavities were formed, apparently by expansion of the central necrotic foci of these tubercles, accompanied by dense infiltration of polymorphonuclear leukocytes, and more or less rapid endobronchial spread of the infection. However, cavities never came to occupy more than half of the initial tubercles even in animals on diet C, and usually were poorly defined spaces, partially filled and surrounded by inflammatory cells. Granulation tissue rarely formed about the cavities or anywhere within the tubercles of animals on diet C, except along the pleural surfaces, and even there but little collagen was produced.

Cavities were found occasionally in the initial tubercles of animals on diet A, evidence that necrosis had occurred here, too. In fact, a majority of the initial tubercles of this diet group undoubtedly underwent more or less central necrosis. But within 150 to 200 days the walls of cavities or the centers of the tubercles in this group had been transformed into pale-staining, eosinophilic "scars" suggesting amyloid, although amyloid could not be demonstrated. These scars contained little collagen, and a majority of the inflammatory cells within them were ill-defined (Fig. 10). Bacilli were difficult to demonstrate in any lesions of animals on diet A after 150 days.

Tubercles of the thoracic lymph nodes and spleens of animals on

diet C often were partially transformed into abscesses by 150 days after infection, i.e., they were densely infiltrated by polymorphonuclear leukocytes and more or less necrotic. In the later stages of the disease, bacilli became increasingly abundant in the lesions (Fig. 11). In animals on diet A, tubercles of the lymph nodes also formed abscesses that apparently persisted without great change from about 130 days after infection to the end of the observation period. At the same time, lesions in the spleens of animals on diet A usually were transformed largely into an eosinophilic substance (Fig. 12). Secondary tubercles developed to about the same degree in the livers of animals on all of the diets, but were largest and persisted longest in animals on diet C (Fig. 13). The livers of animals on diet A at 180 to 200 days often contained poorly defined foci of eosinophilic material that were believed to represent the "scars" of healed tuberculosis (Fig. 14). Amyloid could not be identified within these foci.

Tubercles of other organs of the body rarely developed to macroscopic size, and apparently did not contribute to the outcome of the disease.

TUBERCULOSIS INDUCED BY BACILLI OF LOW VIRULENCE (FIGS. 15 TO 20)

For the purposes of this study, virulence has been measured only by the capacity of bacilli of the H37Rv strain to induce tubercles to develop in the lungs of hamsters to diameters of about 5 mm. within 75 days. However, in one experiment with three diet groups of over 50 animals each, infections were unintentionally induced by bacilli of low virulence. Thus initial tubercles of these animals were less than half the expected size after 75 days, and even at 200 days were rarely more than 5 mm. in diameter.

The significant macroscopic features of tuberculosis induced by these bacilli of lower virulence are illustrated by Figures 15 to 20, which were prepared from animals on diets C (6 per cent protein) and A (30 per cent protein), killed at 75, 125, and 200 days after infection.

Levels of dietary protein apparently did not influence the susceptibility of hamsters to this strain of bacilli, for counts of initial tubercles after 75 and 125 days corresponded closely to the number of bacilli calculated to have been inhaled. Moreover, levels of dietary protein had not influenced the progress of the disease in these diet groups when the experiment was terminated 200 days after infection. Indeed, after developing for 200 days, the size and distribution of the lesions and their histologic appearances were about equal to those of lesions com-

monly found 75 days after infection by inhalation of bacilli of higher virulence. However, bacilli were much less frequent in all sites, and signs of necrosis were exceedingly uncommon in these lesions.

Later experiments with other hamsters of this stock and another culture of the H37Rv strain demonstrated that the results which have been described could not be attributed to a change in the capacity of the animals to acquire resistance. Therefore, they are presented as evidence of a change in virulence of the organism.

Discussion

In these experiments, levels of dietary protein neither altered the susceptibility of Syrian hamsters to inhalation infection by tubercle bacilli (H37Rv strain) of high or low virulence, nor modified the course of tuberculosis caused by organisms of higher virulence until the disease had progressed for upwards of 130 days. Beyond this time, however, tuberculosis of animals that inhaled the more highly virulent bacilli progressed most rapidly in animals on the lowest level of dietary protein. Tuberculosis induced by bacilli of low virulence progressed less rapidly in all diet groups than that induced by bacilli of high virulence. Still, its progress had not been modified appreciably by levels of dietary protein when the experiment was terminated 200 days after infection.

These observations are difficult to reconcile with the results of experiments on nutrition and tuberculosis induced in mice by injecting bacilli intravenously. Diets have modified this disease of mice within 4 weeks.[6,7] Perhaps a comparison of tuberculosis in different species may be impossible or, if not impossible, difficult when infections are induced by different routes and techniques, for these factors must determine, to a very great degree, the relations of hosts and parasites.[12] Thus, tuberculosis induced by inhalation differs widely in its pattern of development from that induced by injection.[14,15,21-25]

It is evident from recent publications that the implications of these differences have not been fully understood.[26] Therefore, a brief review may aid in orienting the results of the present experiments.

Tuberculosis induced by droplet nuclei infection, i.e., by inhalation, develops from separated bacilli deposited at isolated points on alveolar surfaces. Whether few or many bacilli are inhaled, development of a tubercle during the first 4 weeks passes through three distinct phases: (a) progressive growth of bacilli for about 2 weeks in alveolar macrophages that collect about the points at which bacilli have been deposited; (b) then acute inflammatory reactions in and about these

foci, with necrosis of alveolar macrophages and a sharp reduction in the number of bacilli; and (c) transformation of the inflammatory reaction into a less active process which, thereafter, is characteristic of the host-parasite combination (strain of bacillus and species of host animal[14,15]).

Whether few or many bacilli are inhaled, the acute inflammatory reaction 2 to 3 weeks after infection also causes organisms to be carried to the regional lymph nodes and beyond. But when infections are begun with a few initial foci in the lungs, the number of bacilli that are carried to the lymph nodes and beyond during the early stages of disease is always small and often is insignificant. Further spread of the disease within the lungs (and beyond in certain host-parasite combinations) may occur in time, but in all host-parasite combinations examined thus far, later progress of the disease can depend upon activity of a few (at times only one) initial tubercles in the lungs. Obviously, then, one virulent tubercle bacillus can be the infecting dose as well as the source of later progressive disease.

This pattern of tuberculosis (inhalation infection) has been demonstrated in mice, rats, guinea-pigs, hamsters, and rabbits. And by extrapolation, it can be applied readily enough to explain the common forms of pulmonary tuberculosis of infrahuman primates and wild carnivores.[27,28] Indeed, it applies quite as well to pulmonary tuberculosis of man. Therefore, it may not be dismissed as a unique phenomenon, reproducible only in experimental animals by special techniques.

Evidence is now at hand, and will be presented in detail later, to show that levels of dietary protein neither influence susceptibility of guinea-pigs or rats to infection by inhaled tubercle bacilli nor modify the earlier phases of this disease in these animals. Thus, the results of the present study on hamsters are not unique, and levels of dietary protein may be assumed to modify the course of tuberculosis only by modifying a capacity to acquire resistance.[16] However, changes in the pattern and progress of tuberculosis induced by organisms of high virulence became evident only after relatively prolonged intervals (about 130 days).

These observations are difficult to reconcile with current opinions on the nature of resistance to tuberculosis, which have attributed a dominant rôle to monocytes.[29] Indeed, monocytes are said to acquire the capacity to inhibit growth of virulent bacilli within 15 days after the animals had been injected with BCG.[30] Then, more recently, Lurie and associates[31] have presented evidence to show that monocytes of rabbits that had been inbred for resistance are endowed by heredity with a

greater capacity to inhibit multiplication of tubercle bacilli (H37Rv strain) than the monocytes of rabbits inbred for susceptibility. Even so, these "resistant" rabbits developed cavities and endobronchial tuberculosis within 8 weeks, while initial tubercles in the lungs of "susceptible" rabbits were still intact.

In part, this emphasis on the rôle of monocytes in resistance to tuberculosis may reflect the fallacies inherent in attempts to force the patterns of experimental disease induced by inoculation with large numbers of bacilli into correspondence with much more chronic processes. Also, these opinions may reflect the fallacies inherent in some experimental methods.[32,33]

The monocytes undoubtedly have important functions in resistance to tuberculosis, but apparently these functions depend for their development upon experience with infection. Indeed, highly uniform response to inhalation infection by virulent or by relatively avirulent bacilli is compelling evidence against the concept of native or hereditary resistance to tuberculosis.[11,13-15]

Levels of resistance, which are now measurable, develop rapidly when virulent bacilli are inhaled. Rabbits, for example, have resisted inhalation reinfection within 5 weeks after an initial inhalation infection by small numbers of virulent organisms.[11-13] Since the early stages of tuberculosis of rabbits and of hamsters are so closely similar, it may be assumed that hamsters also had developed equal levels of resistance within 5 weeks. But whether or not this is true, the ratio of bacilli to monocytes found in initial tubercles 5 weeks after the more highly virulent bacilli had been inhaled did not change appreciably during the following 6 weeks. Furthermore, in hamsters that had inhaled bacilli of low resistance, the ratio of bacilli to monocytes was established at a lower level, which did not change within 200 days. In either case, constant ratios of parasite to monocyte were established early and maintained for a time. The numerical value of these ratios and the intervals through which they have been maintained apparently depended upon the virulence of the parasite rather than the condition of the host and its level of dietary protein.

The relation of virulence to the immunizing properties of an organism is well known. And in these experiments the one essential difference between the manifestations of tuberculosis induced by organisms of different virulence has been the response to levels of dietary protein. This has been associated with a wide difference in the apparent rate of growth of the organisms even during the earlier phases of the disease. Therefore we suggest that resistance to tuberculosis in the hamster is

developed through at least two steps. The first occurs within the third week after inhaled infection, irrespective of the host and virulence of the parasite.[13,15,34] This endows the monocytes with a capacity to limit, more or less, the growth of bacilli, depending on their virulence. The second step develops much more gradually as the bacilli growing in the expanding tubercles stimulate antibody formation. This occurs most readily when protein is supplied in adequate amounts and the bacilli grow at rates adequate to supply the needed antigens, but not to overwhelm the animal.

Failure of resistance in animals on lower levels of dietary protein may be interpreted to mean that the antigens also can operate to reverse and abolish the first level of resistance. This has occurred most readily when the intake of protein was low and the liver had been badly scarred by tubercles. It has occurred also with higher levels of protein when glomerulosclerosis was far advanced, although the effects of renal damage on tuberculosis were then much less striking.

The first step in developing resistance to tuberculosis in these animals may be attributed directly to a change in the monocytes. The second step demands another mechanism, for now it must protect against the growth of bacilli in extracellular foci as well as within the monocytes. It is in this second stage that dietary protein has been found to be a critical factor in determining the level of resistance.

Summary and Conclusions

Groups of young adult Syrian hamsters were allowed at least 6 weeks for adjusting to experimental diets, after which they were caused to inhale small numbers of tubercle bacilli of human origin and maintained on these diets until killed for study at stated intervals between 75 and 200 days after infection. Three isocaloric diets were used. These supplied approximately 30, 17, and 6 per cent protein.

Levels of dietary protein did not influence susceptibility to inhalation infection by tubercle bacilli of high or low virulence, nor modify the progress of disease induced by organisms of low virulence within 200 days. Moreover, levels of dietary protein did not modify the progress of tuberculosis induced by organisms of high virulence until about 130 days after infection. Beyond this time, however, progress of tuberculosis was most rapid in animals on 6 per cent dietary protein. In hamsters on 30 per cent protein, tuberculosis was arrested, or regressed for a time. Its reactivation in animals on this diet was associated with progressive glomerulosclerosis.

It is concluded that, in hamsters, dietary protein can be a critical

factor in the development of effective levels of resistance to tuberculosis. This requires relatively prolonged interaction of host and virulent parasites, and apparently reflects the combined actions of the monocytes and other factors which, presumably, are antibodies. Rapid failure of resistance in association with hepatic and renal disease supports this suggestion.

It is concluded, also, that the highly uniform reaction of these animals for upwards of 130 days, irrespective of their diet, is further evidence against the concept of native or hereditary resistance to tuberculosis.

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[*Illustrations follow*]

LEGENDS FOR FIGURES

Figures 1 to 14 show lesions of tuberculosis induced by bacilli of high virulence.

Figures 1 to 6. Macroscopic lesions in thoracic lymph nodes and one lung of each animal, and in spleens of representative animals. The thoracic lymph nodes have been cut vertically to the long axis of the body. Usually the left lung, which is not divided into lobes, is shown.

FIG. 1. Litters of 4 and 6 animals on diet C (6 per cent protein) 75 days after infection.

FIG. 2. Random animals on diet A (30 per cent protein) 75 days after infection. Secondary tubercles in the lungs are less numerous than in Figure 1, but are more numerous in the spleens. Variation in the number of secondary tubercles in lungs is greater among litter mates of Figure 1 than among random animals of Figure 2.

FIG. 3. One litter of 7 animals on diet C, 150 days after infection. Lungs and lymph nodes show wide variations in the progress of tuberculosis; the lesions in the spleens are more uniform.

FIG. 4. Random animals on diet A, 180 days after infection; little evidence of secondary spread beyond the lymph nodes.

FIG. 5. One litter of 7 animals on diet C, 180 days after infection; endobronchial and splenic tuberculosis is conspicuous.

FIG. 6. Random animals on diet A, 200 days after infection; progressive disease, usually miliary and associated with glomerulosclerosis.

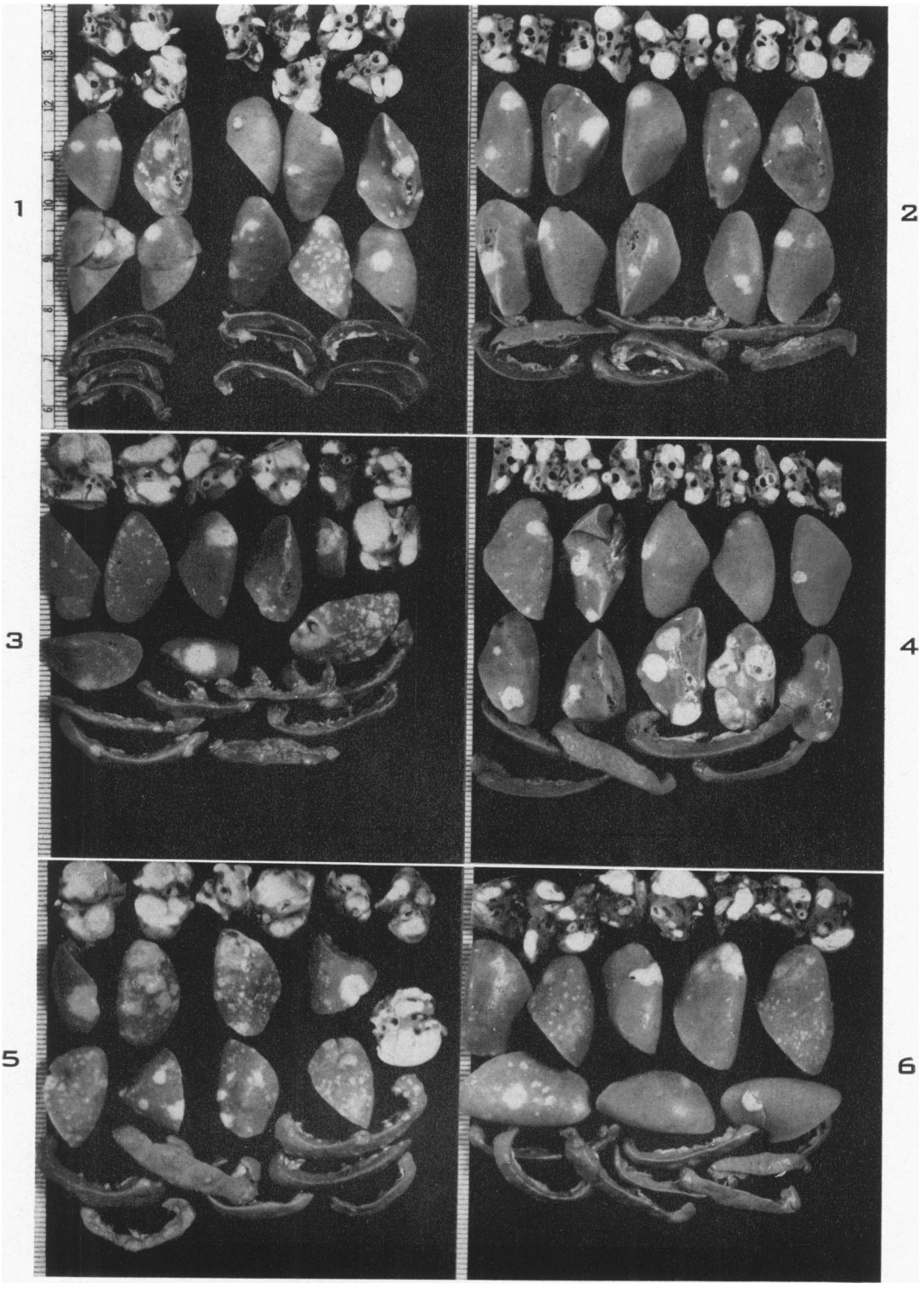
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FIG. 7. The central zone of an initial tubercle from an animal on diet C, 125 days after infection. Alveolar walls are thickened and alveolar spaces and ducts are filled by monocytes and polymorphonuclear leukocytes with necrosis beginning in an alveolar duct. Hematoxylin and eosin stain. × 350.

FIG. 8. The central zone of an initial tubercle from an animal on diet A, 125 days after infection, with exudate in a terminal bronchiole. Hematoxylin and eosin stain. × 350.

FIG. 9. Bacilli in cells about the border of a cavity from an animal on diet C, 180 days after infection. The cavity was partially lined with epithelium. Carbol fuchsin-light green stain. × 800.

FIG. 10. Border of a cavity from an animal on diet A, 200 days after infection. Hyalinized tissue forms the wall of this cavity; a few bacilli are present. Carbol fuchsin-light green stain. × 800.

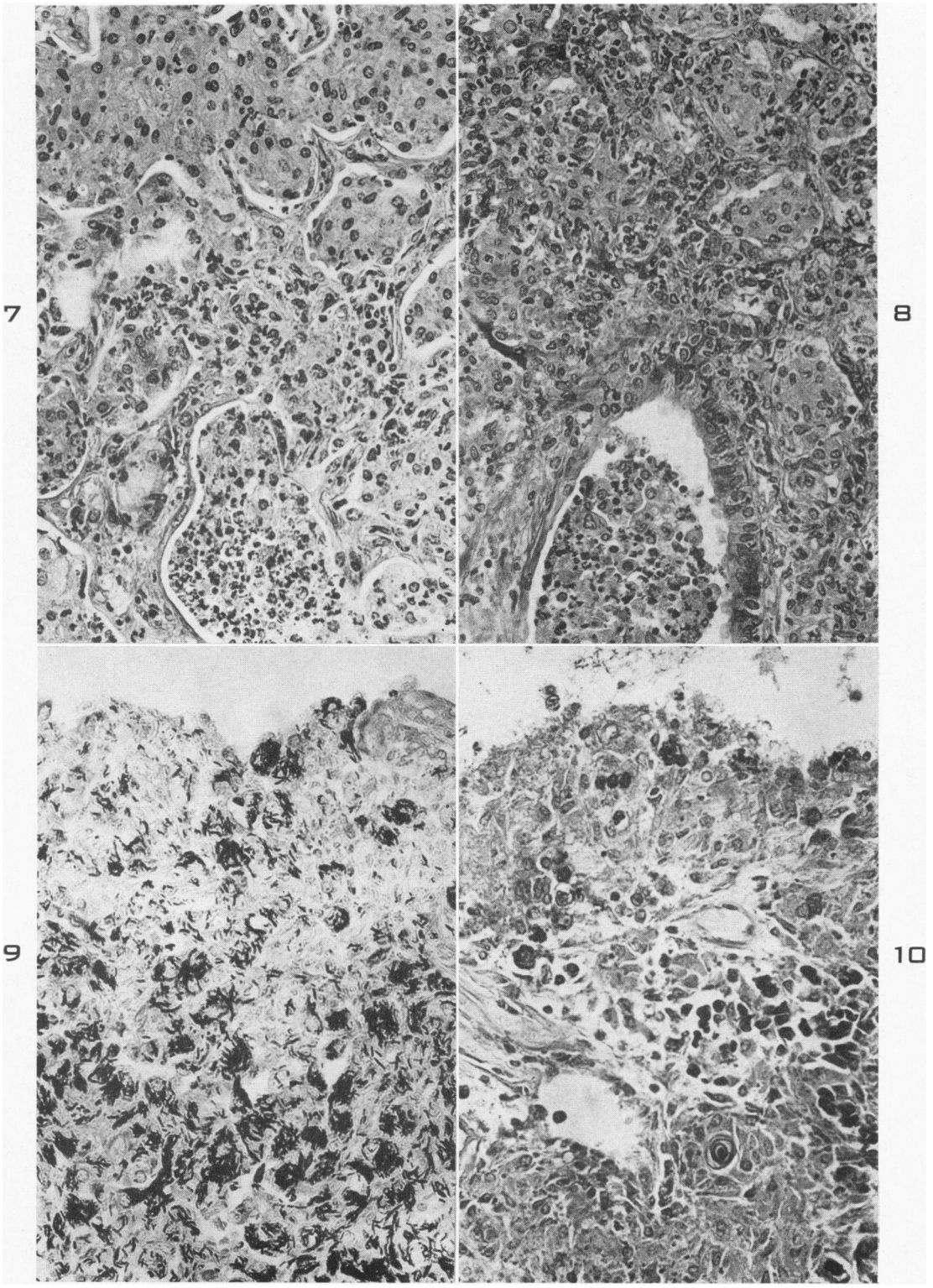
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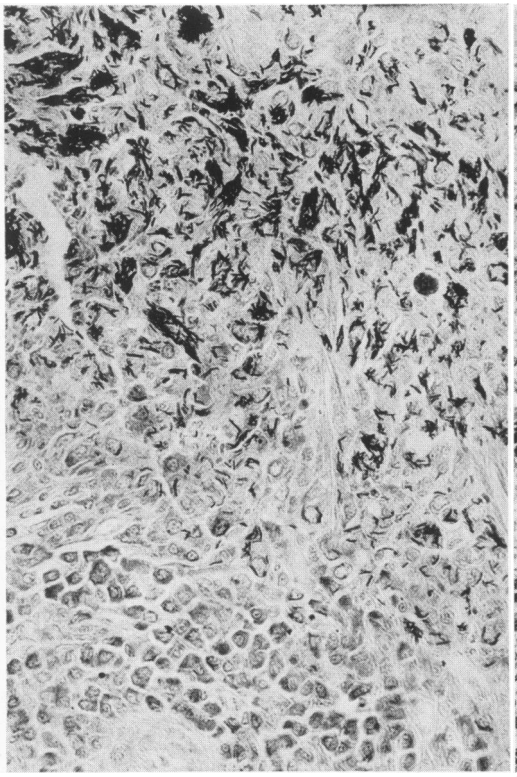
FIG. 11. Bacilli in a splenic tubercle of an animal on diet C, 180 days after infection. Carbol fuchsin-light green stain. × 800.

FIG. 12. Hyalinized tissue in the spleen of an animal on diet A, 200 days after infection. Bacilli are not demonstrated. Hematoxylin and eosin stain. × 800.

FIG. 13. Tubercles at the periphery of a liver lobule of an animal on diet C, 155 days after infection. Hematoxylin and eosin stain. × 250.

FIG. 14. Hyalinized tissue near the periphery of a liver lobule of an animal on diet A, 200 days after infection. Hematoxylin and eosin stain. × 250.



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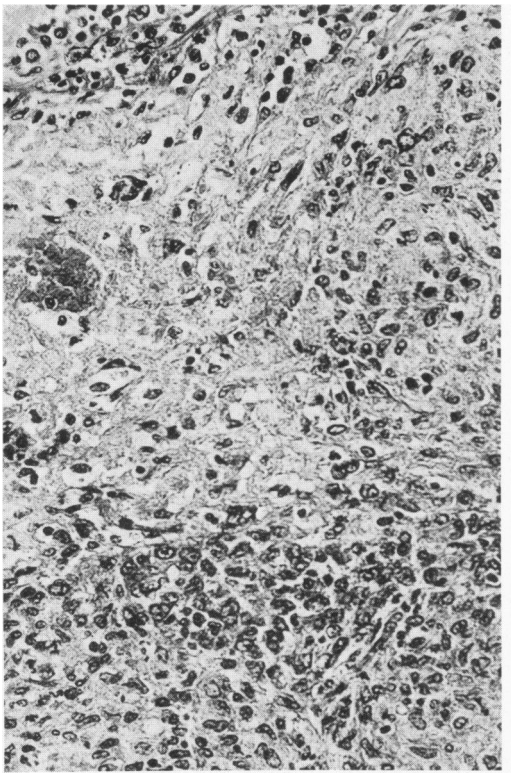

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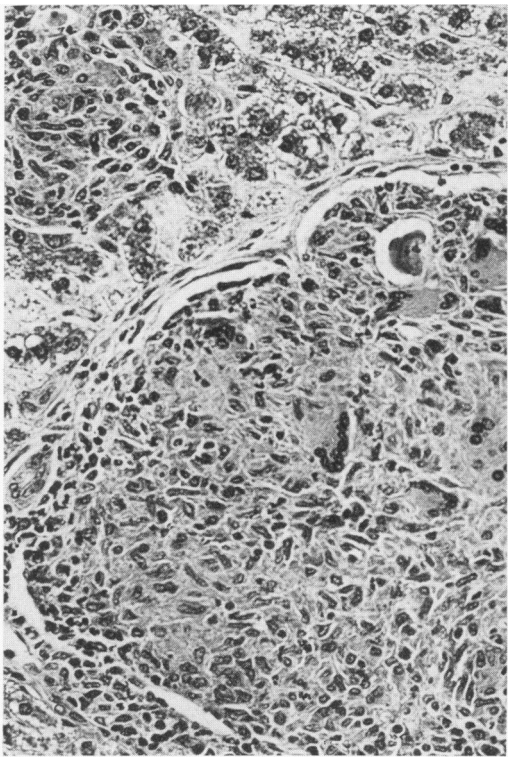

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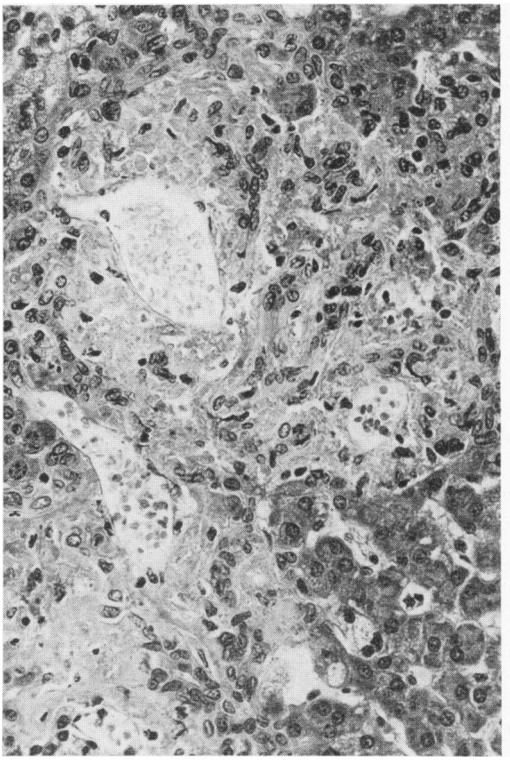

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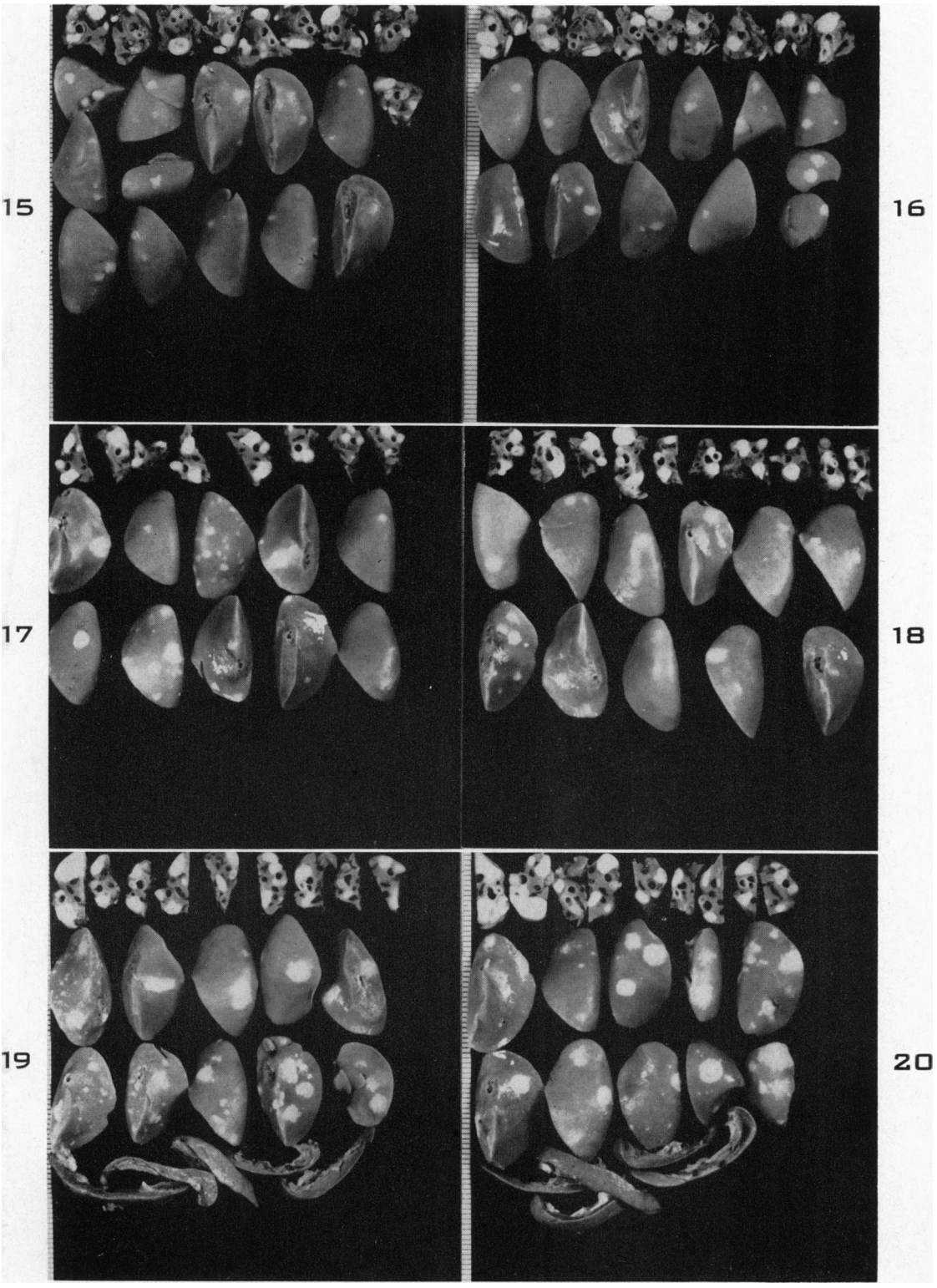
Figures 15 to 20 are from lesions of tuberculosis induced by bacilli of low virulence.

Figures 15, 17, and 19 illustrate tuberculosis in animals on diet C (6 per cent protein); Figures 16, 18, and 20, tuberculosis in animals on diet A (30 per cent protein).

FIGS. 15 and 16. Tubercles of the lungs and thoracic lymph nodes of random animals, 75 days after infection. Spleens are not involved; for comparison with Figures 1 and 2.

FIGS. 17 and 18. Tubercles in lungs and thoracic lymph nodes of random animals. 125 days after infection. Spleens are not involved.

FIGS. 19 and 20. Tubercles in lungs, thoracic lymph nodes, and spleens of random animals, 200 days after infection.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