A Quantitative Pathologic Study of Avian Tuberculosis in the Chick

Effect of Protein and Lysine Dietary Levels

Henry Siegel, M.D., R. L. Squibb, Ph.D., M. Solotorovsky, Ph.D., and W. H. Ott, Ph.D.

ADEQUATE STUDIES in experimental disease require that the state of the host be easily defined in nutritional and biochemical terms and that the disease process be quantitatively and qualitatively defined. As a corollary, disease states must also develop at the proper rate since too slow or too rapid a development may not be useful in maximally demonstrating the influence of certain factors.

In a previous publication,[1] it was suggested that the chick is a desirable host for evaluating host responses and that avian tuberculosis is a disease readily manipulated and easily studied. *Mycobacterium tuberculosis,* avian type, produces a fatal chronic illness in the chick that is characterized by tubercles whose development essentially parallels that in man. Pure genetic lines are readily available in chickens. Their nutritional requirements are well defined, and their growth and behavior are sensitive to changes in physiologic status. Recent studies have demonstrated the ease with which their protein metabolic reactions respond to diurnal rhythms and disease states.[2,3]

Most estimations of an experimental disease state are first approximations and include injections of approximately known amounts of the infectious agent combined with estimations of the disease effects by mortality or gross pathologic changes. Second approximations have included data from studies of inoculations of an exact number of organisms with gross estimations of the disease process and some histologic observations.[4-6] Where techniques are not easily available for the inoculation of an exact number of organisms but only that of a standardized culture, second approximations can be made by quantitating the extent and distribution of the lesions and of their finer histologic structure, or by evalu-

From the Bureau of Biological Research and Department of Bacteriology, Rutgers—The State University, New Brunswick, N. J.; Merck Institute for Therapeutic Research, Rahway, N. J.; and the Department of Forensic Medicine, New York University Schools of Medicine, New York, N. Y.

Supported in part by Grant E-2634 from the National Institutes of Health, U. S. Public Health Service.

Accepted for publication Oct. 3, 1967.

Address for reprint requests: Department of Forensic Medicine, New York University Medical Center, 520 First Ave., New York, N. Y. 10016.

ating the rate of change of a lesion that usually develops in a predictable manner.[7]

The present paper includes the methods employed in the quantitative estimations of the gross and finer pathologic changes evoked by the intravenous injection of known amounts of standardized cultures of avian tubercle bacilli into chicks. Miliary tuberculosis is produced, with the vast majority of the tubercles being found in the liver and spleen. The total tuberculous involvement, both as to number and distribution, can be easily estimated in the liver and spleen, as can the individual morphologic features that characterize the tubercle at various stages. The presence of more than a rare tubercle in other tissues immediately points to the introduction of a new factor. These methods have been used in studying the effects of certain nutritional conditions[8] and of protein metabolism[2,9] in this disease state. Included in the present report are the results of the pathologic studies in five consecutive experiments performed over a period of several years in two separate laboratories and hitherto not reported. The methodology is given in detail in one experiment. A summary of the results of the other experiments is included.

Materials and Methods

Experiment 1

M. tuberculosis, avian type, Kirchberg strain, was used. It had been maintained by weekly serial passage in Dubos-Tween albumin medium interspersed by an occasional passage through the freeze-dry state and an occasional passage in chicks. The cell suspension for infection was a 1:10 dilution (unless otherwise noted) of a 10- to 14-day-old culture adjusted to permit 50–60% transmission of light at 650 mμ with a spectrophotometer.

White Leghorn male chicks, 2 weeks of age, obtained from a commercial hatchery, were used. The birds were housed in an air-conditioned room at a temperature of 70–75° F. Chicks were selected for each group so that the average weights were comparable. All chicks were maintained on their respective experimental diets for 2 weeks before they were infected. The composition of the diets in this experiment is shown in Table 1. These purified diets were adequate in all known nutrients and varied only in the amount of dextrose, protein, and supplementary amino acids. The ratio of supplementary amino acids to protein was kept constant. Control groups were given a commercial chick starter ration containing about 20% protein. Water was given ad libitum. All chicks were weighed weekly, or more often as indicated.

The birds on the restricted diets were housed in individual cages. The average amount of food consumed by the chicks on the low-protein diet (LPD) was fed the next day to each of the birds whose caloric intake was being restricted. The latter birds were on either the normal or the high-protein diets (HPD).

The chicks were infected intravenously with 0.5 ml. of a 1:10 dilution of the standard inoculum. Previous trials had shown that chicks fed the commercial diet and infected at the same age as those presently used would die in 5–9 weeks.

The experiment was terminated at the end of the fifth week of infection. At this time 1 chick had died in most of the infected groups and the infected chicks on the

Table 1. Composition of the Synthetic Diets

Component	Protein in diet (%)		
	Low	Normal	High
Labco casein (vitamin free)	6.7	20.0	47.5
Amino acetic acid	0.667	2.0	4.75
Arginine	0.167	0.5	1.20
Cystine	0.067	0.2	0.475
Dextrose	73.9	58.8	27.6
Constant ingredients*	18.55	18.55	18.55

* Consisted in each diet of (%): calcium gluconate 2.5; potassium dihydrogen phosphate 1.0; cellulose 5.0; salt mixture 5.0 (J Biol Chem 138:459, 1941); wheat germ oil 4.5; choline chloride 0.2; Viadex (A & D) 0.2; inositol 0.1; p-aminobenzoic acid 0.03; niacin 0.01; calcium pantothenate 0.004; pyridoxine 0.002; thiamine 0.002; riboflavin 0.002; medadione 0.0004; folic acid 0.0004; biotin 0.00004; and Vitamin B_{12} 0.000005.

low level of protein in the diet had gained very little weight and were in poor general condition. Estimations of the degree of tissue reaction in the occasional bird that died were not included in the final analysis.

At the end of the experiment all chicks were sacrificed by manual fracture of the cervical spine and spinal cord, and were necropsied. Intercurrent infections were not encountered. There was no gross evidence of edema. The weights of the liver and spleen were recorded and slices were fixed in 10% formalin and in Bouin's fluid. Other tissues were fixed in 10% formalin. The tissue slices were embedded in paraffin and the histologic sections were stained with hematoxylin and eosin.

Studies on pathogenesis revealed that the earliest tissue reaction, regardless of the size of the inoculum, occurred in the liver during the second week. Foci composed of several lymphocytes appeared first and were soon admixed with small epithelioid cells. Rarely, larger lymphocytic foci were present in test and control chicks. Epithelioid foci appeared in the spleen during the third week. The number of tubercles, both lymphocytic and epithelioid, increased in number—whether or not the size of the inoculum was increased—and then remained constant in concentration. By the end of the fourth week all tubercles were epithelioid. The weight of the liver and spleen progressively increased and since the concentration of the tubercles remained constant the total number of tubercles progressively increased. Further progressive changes in the development of the tubercle are described below.

The following criteria for the analysis of the character and the extent of the tissue reactions were used.

Number of Tubercles per Low-power Field. This figure was estimated using wide-plane 10× ocular and 10× objective lenses. Where a smaller tubercle was opposed to a larger one each was counted separately. Each count represents the average of 15 fields.

Area of Tissue Replaced or Displaced by Tubercles as Measured per Low-power Field. A 1+, equivalent to less than 10%; 2+, 10 to 25%; 3+, 25 to 50%; 4+, more than 50%.

Developmental Stage of Tubercles. Not all the lesions in any one organ were at the same stage of development but they were sufficiently uniform to fall into one of the following categories. Splenic tubercles tend to be slightly more advanced than those in the liver of the same bird.

1. Small collections of epithelioid and lymphoid cells (Fig. 1).
2. Tubercles were larger and composed of epithelioid cells and a thin rim of lymphoid cells, histiocytes, and an occasional granulocyte (Fig. 2).
3. The epithelioid cells were fused and vacuolated (Fig. 3).

Table 2. Effects of Dietary Protein Levels on Tissue Reactions of Chicks in Experiment 1

Dietary protein (%)	Food intake	Infection	Average weight (gm.)				Tissue reaction					TB Index
			Start of infection (body)	At sacrifice			Tubercles/LPF		Degree of leukocytic response (liver)	Amyloid deposits (degree)		
				Body	Liver	Spleen	Liver	Spleen		Liver	Spleen	
6.7	Ad lib	No	89	155	6.5	0.2						
6.7	Ad lib	Yes	87	142	11.9	1.1	22	64	1.3	1.2	1.0	332
20.0	Ad lib	No	112	464	14.3	1.3						
20.0	Ad lib	Yes	112	452	24.1	4.2	9	23	2.4	0.4	0.8	314
20.0	Restricted	No	123	238	6.2	0.5						
20.0	Restricted	Yes	120	217	9.2	1.1	23	36	1.2	0.3	0.6	251
47.5	Ad lib	No	126	526	14.1	1.2						
47.5	Ad lib	Yes	126	444	25.2	3.8	15	23	1.4	0.4	0.8	378
47.5	Restricted	No	144	250	5.4	0.4						
47.5	Restricted	Yes	140	257	11.0	1.5	35	43	0.5	0.3	0.3	450
20.0*	Ad lib	Yes	116	430	19.9	3.2	12	29	2.5	0.3	0.6	332
20.0*	Ad lib	Yes	120	401	24.4	3.4	9	26	2.8	0.8	1.4	308

Each group started with 10 chicks.
* Commercial diet.

4. Necrotic changes were present within the tubercle. Amyloid may be present in the outer zone (Fig. 4).

5. The centers of the tubercles were composed of necrotic epithelioid cells bordered by a layer of epithelial syncytia and giant cells. The outer zone was composed of epithelioid cells, histiocytes, and a few fibroblasts, granulocytes, and lymphoid cells. The latter were mainly lymphocytes. Amyloid deposits were almost always present in the outer zone (Fig. 5).

6. At this stage the mature tubercle was present. It was composed of four zones: an inner necrotic zone; a zone of giant cells; a zone of epithelioid cells and histiocytes; and an outer zone of histiocytes, fibrous tissue elements, lymphocytes, granulocytes, and amyloid deposits (Fig. 6).

Leukocytic Reaction. The degree of reaction was estimated by the average number of layers that these cells formed about the tubercles. The cells included lymphocytes, other lymphoid cells, and granulocytes. Most of the cells were lymphocytes. Fibrocytes, macrophages, and epithelioid cells were excluded. Most of the leukocytic cells were present in the outer portion or around the tubercle. For this analysis only the tubercles in the liver were studied. The normal cytologic composition of the spleen precluded a similar analysis. One cell layer was indicated a 1+, two cell layers by 2+ (Fig. 7), three cell layers by 3+, and four or more cell layers by 4+ (Fig. 8).

Amyloid Deposition in the Tubercle (Fig. 9). Amyloid deposits in the outer portion of the tubercle and tends to form a ring. The amount was estimated as follows: 1+, amyloid present in some of the tubercles; 2+, amyloid present to a slight degree in almost every tubercle; 3+, amyloid present in almost every tubercle and in some tubercles sufficient to form a ring in the outer portion; 4+, amyloid present in every tubercle in amounts sufficient to form a ring in the outer portion. This amyloid gives some atypical color reactions.

Tuberculosis (TB) Index

[No. tubercles/*LPF* of liver] [Liver weight (gm.)] +
[No. tubercles/*LPF* of spleen] [Spleen weight (gm.)]

where *LPF* indicates low-power field.

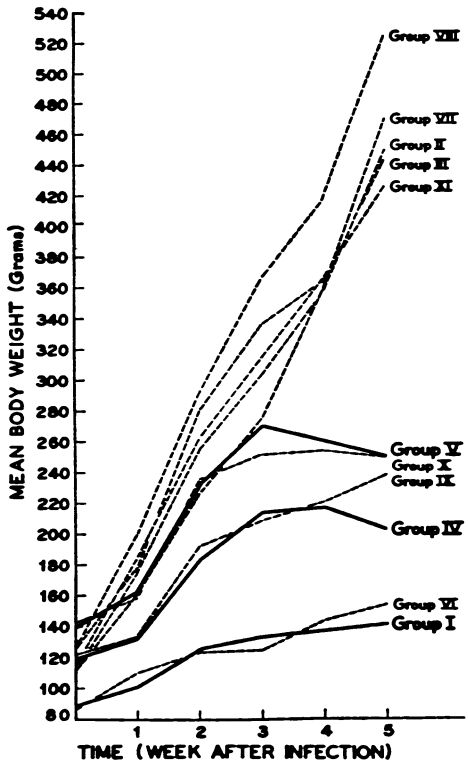
Results

Experiment 1 (Table 2)

Growth Curves (Text-fig. 1). The chicks used in Experiment 1 were grouped as follows:

Group I	Low protein, ad libitum, infected
Group II	Normal protein, ad libitum, infected
Group III	High protein, ad libitum, infected
Group IV	Normal protein, restricted to that of Group I, infected
Group V	High protein, restricted to that of Group I, infected
Group VI	Low protein, ad libitum, not infected
Group VII	Normal protein, ad libitum, not infected
Group VIII	High protein, ad libitum, not infected
Group IX	Normal protein, restricted to that of Group I, not infected
Group X	High protein, restricted to that of Group I, not infected
Group XI	Commercial ration, ad libitum, infected

Of the noninfected groups, the LPD chicks gained weight very slowly,



TEXT-FIG. 1. Growth curves of chicks on various dietary protein levels and infected with *M. tuberculosis*, avian type, Kirchberg strain; and of noninfected and inanition controls (Experiment 1). Groups I–XI are defined in text.

but chicks on the normal and high levels of dietary protein grew at an equal and approximately normal rate. Infection in all the groups produced a minimal depressive effect on the weight curve. The growth curves of Groups IV and IX, whose diets contained normal amounts of protein and whose total food intake was restricted to that of the infected LPD group, rose slowly for the first 4 weeks. The uninfected group then continued to gain weight slowly, but the infected group lost weight. The final average weights, although far below the comparable infected and noninfected groups fed ad libitum, were definitely above that of the LPD group. The growth curves of Groups V and X, whose diet contained the high level of protein and whose feed intake was likewise restricted to that of the infected LPD group, gained weight for the first 3 weeks. Thereafter the weight of the uninfected group remained stationary, whereas the infected group lost weight. Both growth curves were essentially similar to the restricted groups on normal protein levels but were at a higher level at all points.

TB Index. Of those groups fed ad libitum at normal and low protein levels, the indices were essentially similar: 314 and 332, respectively. Re-

stricting the caloric intake at the normal protein level resulted in less tuberculosis, indicated by a lowered index of 251. However, increasing the dietary protein level increased the number of tubercles to an index of 378, and restricting the caloric intake at such high protein levels raised the index even further to 450. A measure of the internal accuracy of the method and the adequacy of the complete synthetic diet is seen in the similar results found in the normal-protein group and the two commercial-diet groups fed ad libitum: 314, and 332 and 308, respectively.

Leukocytic Reaction. The average degree of leukocytic reaction of the tubercles in the livers of the different groups varied inversely with the tubercle count, regardless of the character or amount of the diet. As the tubercle count decreased from 35 to 9, the degree of leukocytic reaction increased from 0.5 to 2.8.

Other Tissue Reactions. The average developmental stage of the tubercles in all the groups was essentially similar. The amount of amyloid in the hepatic and splenic tubercles was slight. The low-protein diet appeared to increase the liver amyloid slightly. The results obtained by estimation of the area (per low-power field) of tissue replaced or displaced by the tubercles essentially paralleled those obtained by the tubercle counts. There was no change in the distribution of the tubercles in the organs or tissues.

Experiments 2–5

Table 3 summarizes the results of all 5 experiments as it pertains to the tuberculosis index. Decreasing the level of protein in the diet and permitting free access to food resulted in a decreased number of tubercles (in 4 of 5 trials), whereas increasing the protein level produced a distinct increase in the number of tubercles (in 3 of 3 trials). Restricting the caloric intake of the birds on a normal level of protein yielded inconclusive results in 3 separate experiments. One trial of restricting a high-protein diet resulted in a higher TB index. In these experiments varying degrees of inanition were achieved by mixing the experimental diet with different amounts of a nondigestible cellulose and permitting free access to it.

Varying the level of dietary lysine produced an interesting effect. When a 1:150 dilution of a culture was used, lysine levels above and below the normal resulted in fewer tubercles, but when a higher concentration (1:50 dilution) of the same culture was used, lysine levels above and below the normal had the opposite effect—a greater number of tubercles were found. The higher the level of lysine in the diet, the greater was the number of tubercles.

Table 3. Effect of Various Factors on Tuberculosis Index

Experiment & TB culture (dilution)	Protein diet				Lysine diet ad libitum		
	Low ad lib	Normal restricted	High ad lib	High restricted	Low (½ N)	High (2 N)	High (4 N)
Experiment 1							
1:10	332 (314) None	251 (314) Lower	378 (314) Higher	450 (378) Higher	—	—	—
Experiment 2							
1:10	212 (383) Lower	484 (383) Higher	—	—	—	—	—
Experiment 3							
1:10	672 (1056) Lower	846 (1056) Lower	—	—	—	—	—
Experiment 4							
1:10	394 (644) Lower	—	803 (644) Higher	—	—	—	—
Undiluted	1796 (2186) Lower	—	3229 (2186) Higher	—	—	—	—
Experiment 5							
1:150	—	—	—	—	122 (172) Lower	135 (172) Lower	—
1:50	—	—	—	—	402 (279) Higher	503 (279) Higher	674 (279) Higher

Number in parentheses is reference TB index; number above it is TB index of test group. None, Lower, and Higher indicate the effect of the factor being tested.

Varying the dietary protein or lysine level or restricting the caloric intake had no constant effect on the developmental stage or size of the tubercle or on the amount of amyloid. The leukocytic reaction about the tubercle, however, is related inversely to the number of tubercles (Table 4). The greater the number of tubercles per low-power field, the less the leukocytic reaction. This relationship holds in each experiment regardless of the condition to which the chick has been exposed with but one exception. When the dietary lysine level was dropped to below normal the degree of leukocytic reaction decreased directly with the tubercle count.

Table 4. Relationship of Tuberculosis Index to Protein and Lysine Dietary Levels in Chicks Fed Ad Libitum

Control group		Test group		Change (%)
Diet	TB index	Diet	TB index	
Normal protein	314	Low protein	332	+6
	383		212	−44
	644		394	−39
	1056		672	−36
	2186		1796	−18
Normal protein	314	High protein	378	+20
	644		803	+25
	2186		3229	+48
Normal lysine	172	½ N lysine	122	−29
	172	2 N lysine	135	−22
	279	½ N lysine	402	+44
	279	2 N lysine	503	+80
	279	4 N lysine	674	+141

Discussion

Avian tuberculosis in the chick is an excellent experimental model for quantitative studies of a chronic disease state even though precise control of the exact number of bacilli to be injected is not yet available. Finer pathologic and biochemical studies can be made. The disease state is produced in a host by a strain of tubercle bacilli naturally pathogenic for that host. The genetic state of the chick can be easily ascertained and its nutritional status can be readily controlled. The developmental pattern of the basic tissue reaction resembles closely that observed in the miliary lesion in man. The lesion is composed principally of epithelioid cells bordered by histiocytes and fibrocytes, and surrounded and somewhat infiltrated by other inflammatory cells. It undergoes necrosis, and the disease is accompanied by tuberculin hypersensitivity. With proper dosage, death results. The rate of development of the disease can be readily manipulated and the easy definability of the extent of each of the factors making up the gross and microscopic tissue alterations permits adequate quantitation.

The TB index, as used in this study, is a measure of the total amount of tuberculous tissue in the body of the chick without regard to the developmental stage or the size of the tubercle. Under the conditions of these experiments, these two factors were not affected. Since the pathologic process is essentially limited to the liver and spleen, only the weights

of these organs are considered in the index. Squibb,[10] and Squibb, Siegel, and Solotorovsky[2] have included body weight as a factor in their considerations of the quantitative disease process. This was necessary in order to properly interpret their biochemical data which were measures of metabolic processes involving many and widely distributed tissues.

The dietary low-protein level resulted in a decrease in the total amount of tuberculous tissue with no change in the rate of development or the size of the basic miliary lesion. This may therefore represent a decreased susceptibility to tuberculosis on the part of the LPD chick host. The greater mortality observed in these infected LPD chicks is probably due to the combined effect of diet and infection since there were no other demonstrable factors present that could have contributed to the death. The functions of the affected organs were apparently not compromised, and there were no intercurrent infections. Cannon[11] has criticized many prior studies on the ground that a true protein-deficiency state was not produced. He maintained that a lowered plasma albumin level is an essential feature of this state. Lowered plasma albumin levels were obtained under our experimental conditions.[12] In one prior quantitative study partially supported by histologic study of the tissues, Ratcliffe and Merrick[5,6] showed that no effect is produced in guinea pigs and hamsters when organisms of low virulence were used. Even when more virulent bacteria were used it required 6 weeks of a preinfection low-protein diet and 10 weeks postinfection dietary treatment to show some effect on the disease process in guinea pigs, and 20 weeks of postinfection diet treatment to show an effect in hamsters. Other studies[13–15] in which mice were used and which report an adverse effect do not include the results of histologic or bacteriologic examinations of the tissues to prove that the lesions were truly tuberculous. Latent pseudotuberculous infections may be easily activated.[16]

A high-protein diet of 47.5% protein resulted in a distinct increase in the total amount of tuberculous tissue without any effect on the rate of development or size of the basic tubercle. This represents an increased susceptibility of chicks on such a diet. In an experiment governed by the precise control of the number of inhaled tubercle bacilli and checked by some histologic observations, Ratcliffe and Merrick[6] showed that in guinea pigs and hamsters a high-protein diet (30% protein) resulted in more rapidly healing secondary lesions at extrapulmonary sites. Others,[13,14] using mice, have shown that 30% protein has a beneficial effect, but that 40% has a detrimental effect. Hedgecock[14] also showed that varying the source of dietary fat could nullify this result. These mice lesions, though probably tuberculous because of the overwhelming inoculum, were not confirmed histologically and bacteriologically.

In a first experiment varying levels of lysine were tried in an effort to determine whether one or more amino acids were the essential factor or factors governing the effects of the dietary protein levels. When the total number of tubercles was low, both low and high levels of lysine in the diet resulted in fewer tubercles. When the total number of tubercles was higher, low and high levels of lysine had an opposite effect—they increased the number of tubercles above those found in chicks on the normal dietary lysine level. Thus, the effect of lysine is dependent on the size of the inoculum.

When the data in Table 3 are recalculated so as to relate the percentage change of the TB index of the LPD groups from the normal protein group an interesting relationship appears (Table 4). Four of the five results show a progressive decrease as the TB index increases. Expressed in another way: as the number of tubercles decrease, the LPD effect becomes more marked. Conversely, the three HPD trials showed a progressive percentage increase as the TB index increased. As the number of tubercles decreased the HPD effect became less marked.

The lysine experiment may begin to offer a partial explanation of the effects of varying the protein levels in the diet. With low numbers of inoculated bacteria, levels of lysine in the diet above and below the normal resulted in decreasing numbers of tubercles. With the inoculation of a greater number of bacteria, another effect was noted: there was a progressive percentage increase of the number of tubercles as the level of lysine was increased. Thus, in chicks on various dietary protein or lysine levels, the size of the inoculum determines the degree and type of susceptibility to the tubercle bacilli. The reason for this change in susceptibility is not apparent.

In all the experiments except the last there was evident an inverse relationship between the tubercle count and the degree of leukocytic reaction (Table 5). This led to an easy working hypothesis that the tubercle count was a measure of the virulence of the infecting agent and that the decreased leukocytic response associated with the higher counts represented a measure of the decreased reactivity of the host. However, the depressive effect of the low dietary lysine level on the leukocytic reaction noted in Experiment 5 requires some modification of this simplistic explanation. Since lymphocyte emigration to the site of inflammation is dependent on normal purine metabolism and is interfered with by such antimetabolities as 6-mercaptopurine, actinomycin D, and puromycin,[17] it follows that lysine may be involved in purine metabolism. Thus, deficient dietary lysine levels may interfere with purine metabolism which in turn will result in a decreased lymphocytic response. That the pituitary or adrenal cortex may be involved in this effect cannot be discounted.

Table 5. Relationship of Tubercle Count to Degree of Leukocytic Reaction in Liver Tubercles

Factors				
Diet	Diet intake	Culture dilution	Tubercle count	Leukocyte reaction (degree)
EXPERIMENT 1				
High protein	Restricted	1:10	35	0.5
Normal protein	Restricted	1:10	23	1.2
Low protein	Ad lib	1:10	22	1.3
High protein	Ad lib	1:10	15	1.4
Stock	Ad lib	1:10	12	2.5
Normal protein	Ad lib	1:10	9	2.4
Stock	Ad lib	1:10	9	2.8
EXPERIMENT 2				
Normal protein	Restricted	1:10	23	0.5
Low protein	Ad lib	1:10	16	0.6
Normal protein	Ad lib	1:10	12	0.7
EXPERIMENT 3				
Low protein	Ad lib	1:10	65	0.5
Normal protein	Restricted	1:10	63	0.6
Normal protein	Restricted	1:10	56	0.8
Normal protein	Ad lib	1:10	30	0.8
EXPERIMENT 4				
Low protein	Ad lib	Undiluted	55	0.9
High protein	Ad lib	Undiluted	52	0.8
Normal protein	Ad lib	Undiluted	39	1.5
Normal protein	Ad lib	1:10	14	2.6
High protein	Ad lib	1:10	14	2.1
Low protein	Ad lib	1:10	13	2.9
EXPERIMENT 5				
4 N lysine	Ad lib	1:50	31	1.2
½ N lysine	Ad lib	1:50	26	0.9
2 N lysine	Ad lib	1:50	24	1.6
1 N lysine	Ad lib	1:50	15	2.0
1 N lysine	Ad lib	1:150	10	2.1
2 N lysine	Ad lib	1:150	8.6	2.2
½ N lysine	Ad lib	1:150	7.5	1.0

Summary

A method for the quantitative pathologic study of avian tuberculosis in the chick is described.

The effects of varying the protein and lysine levels in the diet were studied. Low-protein diets decreased the susceptibility to the tubercle bacilli. As the size of the inoculum was decreased, the effect of the low-protein diet became more marked. Contrariwise, high protein dietary levels increased the susceptibility of the host and this effect became more marked as the size of the inoculum was increased.

The lysine level in the diet may offer a partial explanation of the protein effects. With low numbers of inoculated bacteria, abnormal levels of lysine decreased the susceptibility of the host. Higher levels of inocula resulted in an increased susceptibility of the host with the degree dependent on the lysine level.

Low lysine levels also decreased the lymphocytic component of the individual miliary tubercle perhaps through altered purine metabolism.

References

1. Solotorovsky, M., Siegel, H., and Ott, W. H. The use of avian tuberculosis in the chick for experimental studies. *Ann NY Acad Sci 52*:696–702, 1949.
2. Squibb, R. L., Siegel, H., and Solotorovsky, M. Protein metabolism in livers of chicks fed deficient-to-excess quantities of protein and lysine and infected with tuberculosis. *J Nutr 86*:133–142, 1965.
3. Squibb, R. L. Diurnal rhythms of tissue components related to protein metabolism in normal and virus-infected chicks. *J Nutr 90*:71–75, 1966.
4. Robinson, H., and Siegel, H. Influence of B vitamins on resistance of rats to induced pneumococcal lobar pneumonia. *J Infect Dis 75*:127–133, 1944.
5. Ratcliffe, H. L., and Merrick, J. V. Tuberculosis induced by droplet nucleii infection: Its developmental pattern in hamsters in relation to levels of dietary protein. *Amer J Path 33*:107–129, 1957.
6. Ratcliffe, H. L., and Merrick, J. V. Tuberculosis induced by droplet nuclei infection: Its developmental pattern in guinea pigs and rats in relation to dietary protein. *Amer J Path 33*:1121–1135, 1957.
7. Robinson, H., Phares, H., Siegel, H., and Graessle, O. Comparative effect of indomethacin and hydrocortisone on experimental tuberculosis in mice. *Amer Rev Resp Dis* In press.
8. Solotorovsky, M., Squibb, R. L., Wogan, G. N., Siegel, H., and Gala, R. The effect of dietary fat and vitamin A on avian tuberculosis in chicks. *Amer Rev Resp Dis 84*:226–235, 1961.
9. Squibb, R. L., Siegel, H., Lyons, M. M., and Solotorovsky, M. Nucleic and free amino acid response in avian tissues during tuberculosis infection. *Poultry Sci* In press.
10. Squibb, R. L. Nutrition and biochemistry of survival during Newcastle disease virus infection: II. Relation of clinical symptoms and starvation to nucleic and free amino acids of avian liver. *J Nutr 82*:422–426, 1964.

11. CANNON, P. Protein metabolism in relation to resistance to infection. Symposia on Nutrition of the Robert Gould Research Foundation, YOUMANS, J. B., Ed., 1950.

12. WOGAN, G. N., SOLOTOROVSKY, M., SQUIBB, R. L., and SIEGEL, H. The serum protein and lipoprotein response to tuberculosis in chicks fed various levels of dietary fat. *Amer Rev Resp Dis 84:*236–241, 1961.

13. LAYTON, H. W., and YOUMANS, G. P. Effect of dietary factors upon the resistance of albino mice to experimental infection with Mycobacterium tuberculosis. *J Bact 90:*958–964, 1965.

14. HEDGECOCK, L. W. Effect of dietary fatty acids and protein intake on experimental tuberculosis. *J Bact 70:*415–419, 1955.

15. DUBOS, R. J., and SCHAEDLER, R. W. Effect of dietary proteins and amino acids on the susceptibility of mice to bacterial infections. *J Exp Med 108:* 69–81, 1958.

16. FAUVE, R. M., PIERCE-CHASE, C. H., and DUBOS, R. J. Corynebacterium pseudotuberculosis in mice: II. Activation of natural and experimental latent infections. *J Exp Med 120:*283–304, 1964.

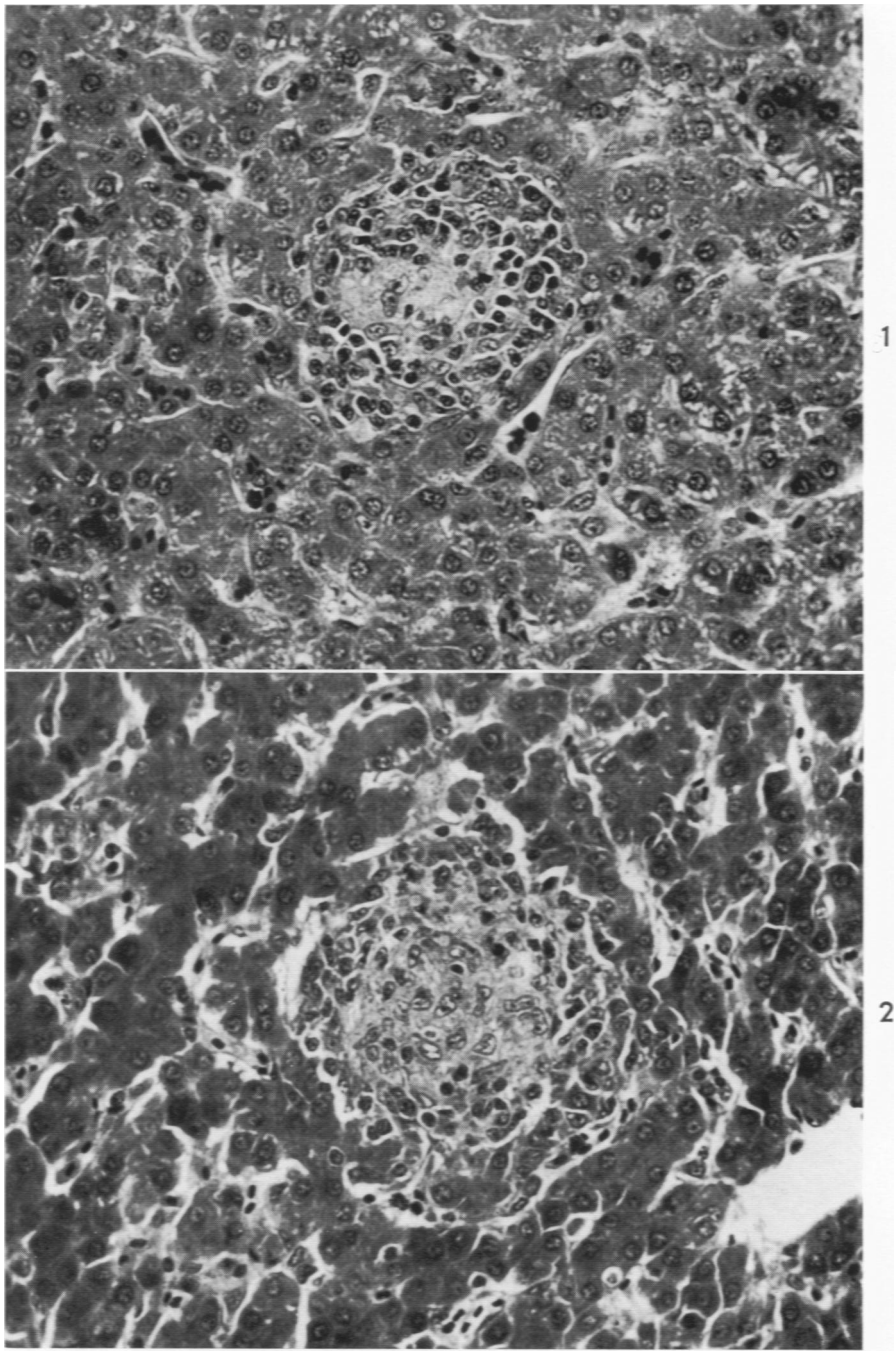
17. PAGE, A. R. Inhibition of the lymphocyte response to inflammation with antimetabolites. *Amer J Path 45:*1029–1044, 1964.

Legends for Figures

All sections illustrated were stained with hematoxylin and eosin.

Fig. 1. Developmental Stage 1 of tubercle in chick liver. Small collections of epithelioid and lymphoid cells. × 400.

Fig. 2. Developmental Stage 2 of tubercle in chick liver. Tubercles are larger and composed of epithelioid cells and a thin rim of lymphoid cells, histiocytes, and an occasional granulocyte. × 400.



1

2

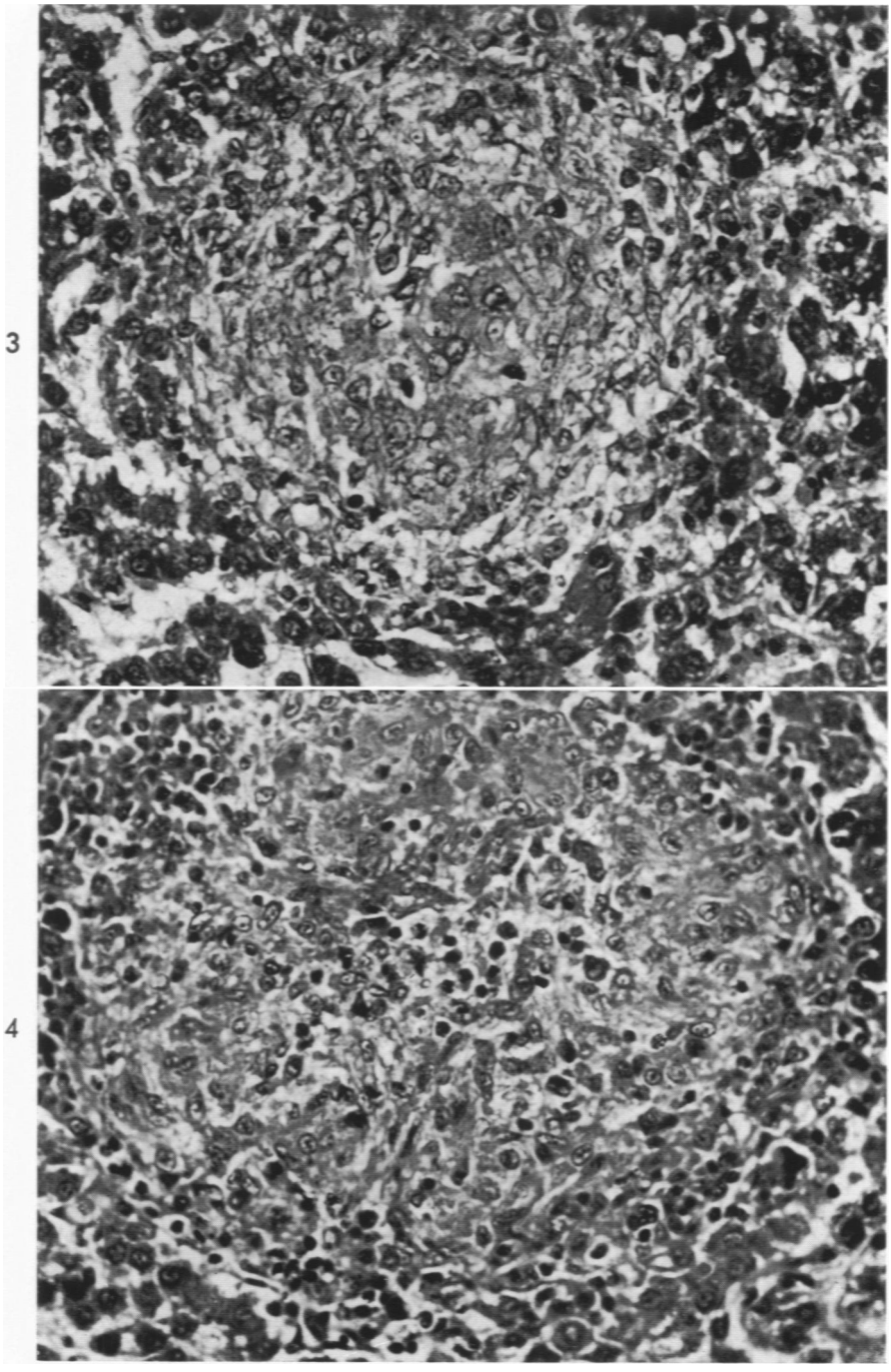


Fig. 3. Developmental Stage 3 of tubercle in chick liver. Tubercles are larger. Epithelioid cells are fused and vacuolated. × 400. **Fig. 4.** Developmental Stage 4 of tubercle in chick liver. Necrotic changes are present within tubercle. × 400.

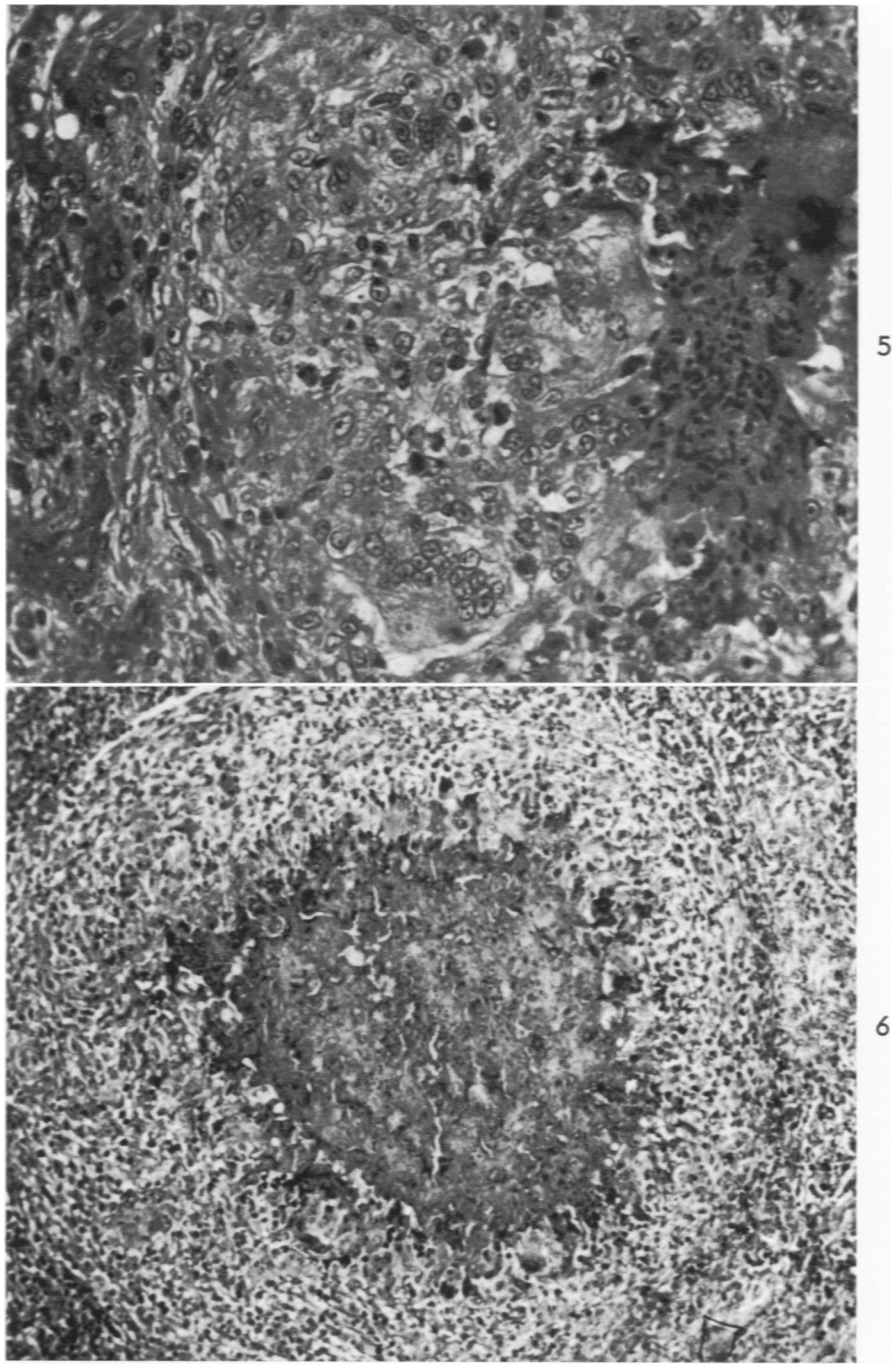


Fig. 5. Developmental Stage 5 of tubercle in chick liver. Figure shows ⅔ diameter of tubercle. Neocrotic center of tubercle, at side, is bordered by layer of epithelioid syncytia and giant cells. Outer zone includes epithelioid cells, histiocytes, some fibroblasts and granulocytes, and lymphoid cells, mainly lymphocytes. Some amyloid is present. × 400.
Fig. 6. Developmental Stage 6 of tubercle in chick spleen. Mature tubercle with 4 zones: inner necrotic zone, zone of giant cells, zone of epithelioid cells and histiocytes, and outer zone of histiocytes, fibrous tissue elements, lymphocytes, granulocytes, and amyloid. × 200.

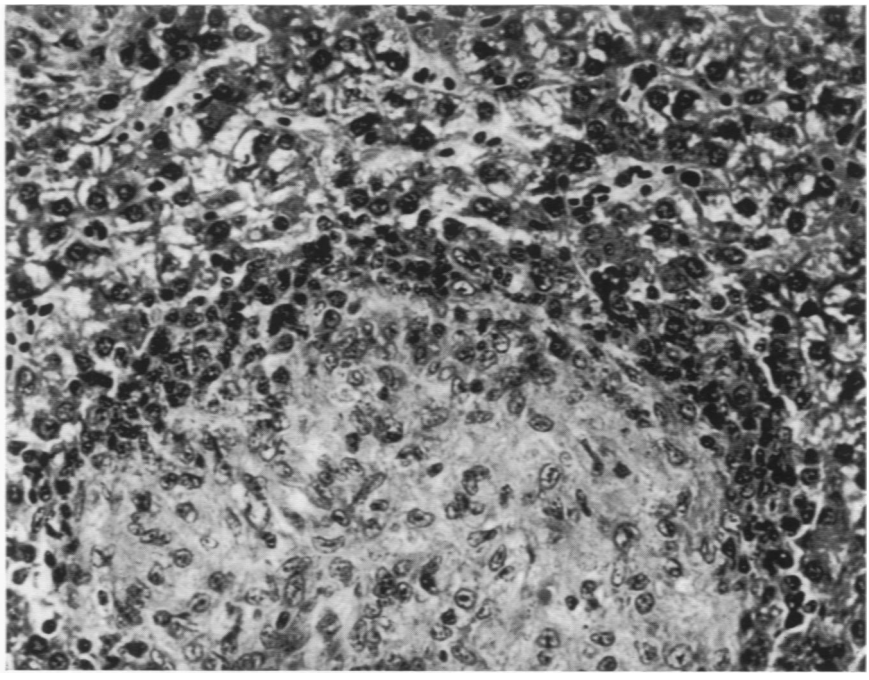
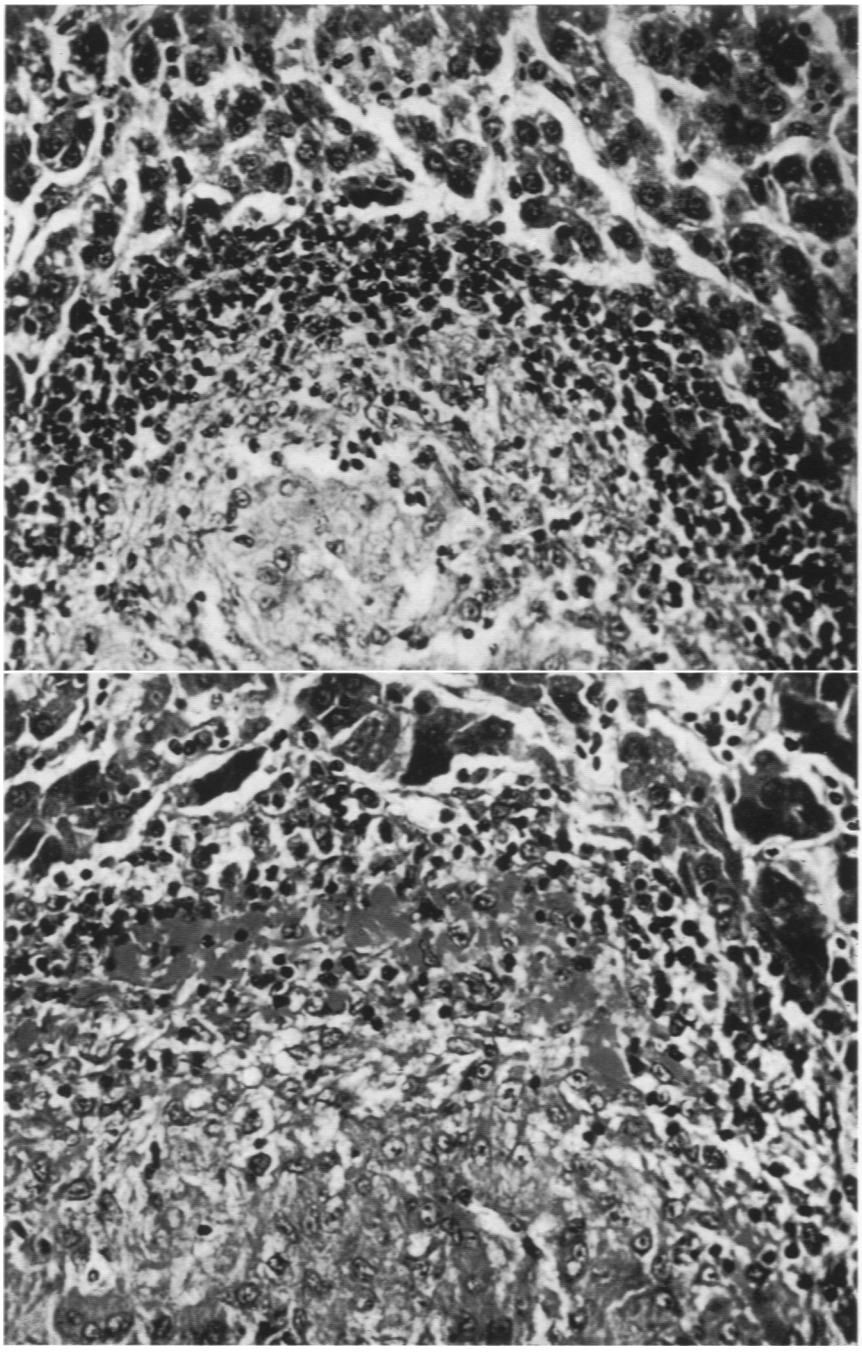


Fig. 7. Two-plus (2+) degree of leukocytic reaction in tubercle in chick liver. Total number of lymphocytes, other lymphoid cells, and granulocytes in outer zone can form 2-cell layer. × 400. **Fig. 8.** Four-plus (4+) degree of leukocytic reaction in tubercle in chick liver. Total number of lymphocytes, other lymphoid cells, and granulocytes in outer zone can form 4-cell layer. × 400. **Fig. 9.** Amyloid deposition in outer portion of chick tubercle. × 400.



8

9