EFFECT OF METABOLIC FACTORS ON THE SUSCEPTIBILITY OF ALBINO MICE TO EXPERIMENTAL TUBERCULOSIS

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Clinical and epidemiological observations strongly suggest that the resistance of man to tuberculosis can be decreased by physiological disturbances resulting from causes as varied as malnutrition, uncontrolled diabetes, or subtotal gastrectomy. There are many reasons to believe that this adverse influence on host-parasite relationship is the outcome of changes in the metabolism of infected tissues, rather than in their immunological state—humoral or cellular. It is known, for example, that resistance to infection in starved or diabetic individuals usually becomes normal again when these patients are returned to a satisfactory nutritional state, or when their diabetes is brought under control by adequate insulin therapy. It appears that susceptibility to infection in these cases is the expression of a reversible disorder, probably biochemical in nature.

There have been published many reports showing that the resistance of experimental animals to tuberculosis can be decreased by feeding them diets deficient in one or several nutritional factors—particularly proteins and certain vitamins (1–11). The present study has a different purpose, not being primarily concerned with the role played in tuberculosis by specific nutritional deficiencies. Rather it is part of an attempt to determine the effect of changes in the physicochemical environment *in vivo* on the fate of microbial pathogens, and hence on the course of infectious processes. This study—which involves experimental infections caused by several bacterial species—has been pursued along two independent but related approaches. On the one hand, experiments *in vitro* have dealt with the effect exerted on the multiplication and survival of microorganisms by substances and conditions which are presumed to occur at the site of the lesion. On the other hand, efforts have been made to modify the resistance of mice to infection by procedures designed to alter their metabolic processes. The present paper deals with the latter phase of this program.

The design of the *in vivo* experiments was much influenced by results obtained *in vitro*. It has been found that the addition of sodium lactate to artificial culture media is inimical to tubercle bacilli, particularly at the slightly acidic reactions known to prevail in inflammatory areas, whereas

several polycarboxylic acids and keto compounds favor the multiplication and survival of the bacilli under the same conditions (12–14). It appeared possible therefore that physiological disorders could affect resistance to infectious diseases through the pathological production of certain metabolites, concentrated perhaps at the site of the inflammatory response. (For a more extensive presentation of this hypothesis, see reference 15.) The present report is limited to a description of some effects exerted on mouse tuberculosis by treatments and nutritional regimens presumed to decrease the glycogen stores of the body, to interfere with the glycolytic activity of tissue cells, or to increase the concentration of acetone bodies and polycarboxylic acids in the body fluids.

EXPERIMENTAL

Materials and Methods

The experiments were carried out with four virulent and six attenuated strains of tubercle bacilli, of human or bovine origin. As the pattern of results was the same in all cases, it will suffice to describe the findings with two strains, one representative of the virulent, and one of the attenuated group: MV, a bovine strain (Vallée) maintained in a state of high virulence for white mice by frequent recovery from the spleens of these animals 2 weeks after infection; BCG-P, a strain of BCG obtained through the courtesy of Dr. J. Aronson, of the Henry Phipps Institute in Philadelphia. The cultures were grown at 37°C. in tween-albumin medium, and were used when 10 to 12 days old.

The animals were albino mice bred at the Rockefeller Institute. They were received in the laboratory the week after weaning, at 4 weeks of age. They were kept in groups of 2 (except as noted) in metal cages on a wire screen without bedding throughout the duration of the experiments. Efforts were made to minimize variations in temperature in the animal room, but it was only during the last 4 months of experimentation that air conditioning of the room was available. The temperature was maintained from then on at approximately 78°F.

The diets were as indicated in each individual experiment. When "pellets" were used, they were Purina laboratory chow, obtained from Ralston Purina Company. According to the producer, these pellets contain 25 per cent protein and 6 per cent fat. Some experiments were carried out with the synthetic diet 191 (16) which contains 18 per cent casein and 5 per cent fat. The other experimental diets consisted of mixtures of dried skim milk (containing 36.8 per cent protein and 0.8 per cent fat) and wheat flour to which other ingredients were added in the desired proportions.

In the case of both synthetic diet 191, and the skim milk–wheat flour diets, 1 kg. of the dry food mixture was resuspended in 1 liter of 7.5 per cent gelatin dissolved in tap water at 45°C. The food-gelatin suspension was allowed to set in a cold room and was preserved there until used. Fresh food was prepared every week.

Food was given *ad lib.*, unless otherwise noted. It was supplied in the form of pellets, or of solid gelatin cakes cut just before use. The temperature of the animal room was such that the gelatin cakes remained set while in the animal cage.

All food remnants were removed, and cages cleaned, once weekly.

In general, the mice were infected within 10 days after weaning, *i.e.*, at approximately 29 to 32 days of age; their weights at that time averaged 15 to 18 gm. Infection was always by the intravenous route, the inoculum being diluted in a final volume of 0.2 ml. of 0.1 per cent bovine albumin in 0.8 per cent NaCl. The infective dose was 0.2 ml. of culture for BCG-P and varied as noted from 0.1 to 0.001 ml. for MV.

Validity of the Results.—Because of the very large numbers of animals used, it was rarely possible to carry out autopsies or cultural tests to confirm the cause of death. In practically all cases however, and in particular with the dietary regimens which did not allow for maximum growth of the mice, groups of 6 to 10 animals on each experimental diet were kept uninfected for the duration of the tests in the same room and under exactly the same conditions as the infected ones. The results of experiments in which deaths occurred in the uninfected control groups have not been used in the present report.

The experiments were carried out with groups of 10 to 20 animals. In general, the differences in survival times between groups were so striking as to give a high degree of significance to the results. However no attempt was made to assess the findings in statistical terms. Instead, all experiments were repeated numerous times (at least 3 and up to 10 times). The results obtained were considered significant—and have been reported here—only in cases in which all experiments without exception gave differences which were obvious and of the same order of magnitude.

Effect of Limiting the Amount of Food:

In general, the effect of malnutrition on experimental tuberculosis has been studied by the use of diets deficient in some essential growth factor—particularly vitamins or protein. None of the diets used in the following experiments was grossly deficient in any of the essential dietary factors. The regimens were designed to establish, not deficiency states, but rather different levels of undernutrition—produced either by short periods of acute fasting or by a chronic shortage of food. The effect of these two kinds of regimens will now be considered separately.

The response of tuberculous mice to acute but transient fasting was studied by depriving the infected animals completely of any source of food for periods of 30 consecutive hours.

Mice were given *ad lib.* either pellets, or the synthetic diet 191, or a mixture of wheat flour (66), skim milk (33), and salt (1). Duplicate groups of animals were also fed these diets *ad lib.* except that the food was entirely removed from their cages for periods of 30 hours on the 4th, 11th, and 18th day after infection (water remained available all the time *ad lib*). In other words, the animals in this second group were fasted for 30 hours every week. Ten mice in each of the groups were infected with 0.01 ml. MV. Their survival time is presented in Table I and Fig. 1, which summarize the results of four consecutive experiments carried out over a total period of 5 months. Table I also indicates the weight gain of uninfected mice kept on the experimental regimens for 3 weeks (average of ten mice).

As appears from the results presented in Table I and Fig. 1, mice fed *ad lib.* without interruption survived as a group much longer than those also fed *ad lib.* except that they were deprived of food for 30 consecutive hours every week. The results were essentially the same whether the animals received pellets, or a skim milk–wheat flour mixture, or synthetic diet 191.

The preceding experiments dealt with the effect of *acute* but transient fasting on resistance to tuberculosis. In contrast, the following tests were designed to determine the survival time of tuberculous mice maintained in a *chronic* state of undernutrition throughout the period of observation.

TABLE I

(see Fig. 1)

Effect of Acute Fasting on Mouse Tuberculosis

Fig. No.	Regimen	Weight gain*	Cumulative number of mice (out of 10) dead at					
			3 wks.	4 wks.	5 wks.	6 wks.	7 wks.	8 wks.
		gm.						
1-a	Skim milk 33, wheat flour 66, *ad lib.*, uninterrupted	6.2	0	1	5	10		
1-as	Skim milk 33, wheat flour 66, *ad lib.*, withdrawn 30 hrs. weekly	4.5	4	7	9	10		
1-b	Pellets, *ad lib*, uninterrupted	9.8	0	1	3	4	7	
1-bs	Pellets, *ad lib.*, withdrawn 30 hrs. weekly	5.7	3	5	5	8	10	
1-c	Pellets, *ad lib.*, uninterrupted	8.4	0	1	1	3	6	10
1-cs	Pellets, *ad lib.*, withdrawn 30 hrs. weekly	4.2	0	2	2	6	10	
1-d	Synthetic diet 191, *ad lib.*, uninterrupted	Not weighed	3	5	6	7	10	
1-ds	Synthetic diet 191, *ad lib.*, withdrawn 30 hrs. weekly	Not weighed	3	7	8	8	10	

* Average of 10 uninfected mice over a total period of 3 weeks.

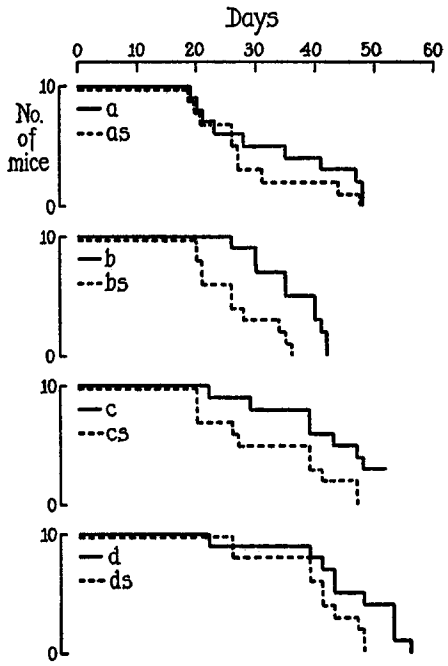

FIG. 1. Effect of fasting on mouse tuberculosis. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table I.

When food was supplied *ad lib.*, the average daily consumption per mouse (30 to 40 days of age) was found to be slightly less than 5 gm. of Sherman diet, a mixture consisting of: whole wheat flour (66), dried whole milk (33), NaCl(1). On this regimen (with water supplied *ad lib.*,) the animals gained approximately 8 to 10 gm. weight during the first period of 3 weeks. The effect exerted by limitation of the quantity of food on susceptibility to infection was determined by the following technique:

Mice were kept singly in metal cages. Some were fed the Sherman mixture *ad lib.* Others received 3.8 gm. of it daily, corresponding to approximately 75 per cent of the maximum food intake. The animals on the restricted regimen gained little if any weight during the 2 months of observation, but otherwise appeared healthy. A third group received Sherman diet *ad lib.* except that the food was entirely removed from their cages for periods of 30 hours on the 4th, 11th, and 18th day after infection (water remained available all the time *ad lib*).

Groups of 10 animals each were infected with 0.01 ml. of the culture MV, 2 days after being placed on either of the three food regimens (Table I A).

The mean survival time was 27.4 days (23 to 40) for the mice fed *ad lib.*, and 28.7 days (19 to 43) for those receiving only 75 per cent of the maximum

TABLE 1 A

Effect of Chronic Undernutrition on Mouse Tuberculosis

Regimen	Weight gain*	Cumulative number of mice (out of 10) dead at				
		3 wks.	4 wks.	5 wks.	6 wks.	7 wks.
	gm.					
Sherman diet *ad lib.*, uninterrupted.....	9.8	0	6	9	10	
Sherman diet *ad lib.*, withdrawn 30 hrs. weekly.....	5.8	4	10			
Sherman diet 3.8 gm. daily, uninterrupted..	1.0	1	6	9	9	10

* Average of 10 uninfected mice over a total period of 3 weeks.

food intake. The results are presented in Table I A. Thus, there was no evidence that the susceptibility of mice to tuberculous infection was modified to an appreciable extent by limitation of the food intake, even though it was of such a degree as to reduce drastically the increase in weight of uninfected animals.

Effect of Content of Diet in Skim Milk:

One of the nutritional factors most commonly assumed to play an essential role in resistance to tuberculosis is the protein content of the food. In none of the tests reported here was the percentage of protein the only variable in the composition of the diet. However, its content in skim milk was varied from 4 to 33 per cent in several experiments designed for other purposes. The weight balance in the diet was then made up either with wheat flour, cerelose, peanut oil, sodium citrate, or mixtures of these materials.

Table II presents the results obtained (in three consecutive experiments) with some of these mixtures in comparison with those obtained with synthetic

diet 191 or with pellets. Food and drinking water were provided *ad lib.* The mice (in groups of 10) were infected with 0.01 ml. of culture MV the morning before being placed on the experimental diets. Other groups of 6 were kept as uninfected controls and weighed every week. The average weight gain per mouse for the first 3 weeks in each non-infected group is recorded in Table II, as well as the time of death of the infected animals.

The results of the three experiments presented in Table II show that, under the conditions of the tests, mice fed diets containing only 4 or 5 per cent skim milk (supplemented with peanut oil) were no more susceptible to tu-

TABLE II

Effect of Concentration of Protein and Fat in the Diet on Mouse Tuberculosis

Percentage composition of diets*				Weight gain	Cumulative number of mice (out of 10) dead at				
Skim milk	Wheat flour	Cerelose	Peanut oil		1 wk.	5 wks.	7 wks.	9 wks.	11 wks.
				gm.					
4	45	30	20	3.8	0	1	2	3	3
4	45	50	—	3.0	1	7	10		
34	65	—	—	8.8	0	0	4	4	4
5	74	—	20	11.0	0	0	1	3	8
5	74	20	—	8.2	0	0	3	7	10
15	64	—	20	10.1	0	0	1	4	6
15	64	20	—	10.8	0	0	2	8	8
33	66	—	—	10.1	0	0	1	6	7
5	74	20		4.6	1	2	8	9	9
Synthetic diet 191 (18 per cent casein, 5 per cent fat).....				8.4	0	1	6	9	9
Pellets (24 per cent protein, 5 per cent fat).....				9.6	0	3	8	8	9

* 1 per cent salt added.

berculosis than those receiving either pellets (containing 25 per cent protein), or diets containing 15, 33, or 34 per cent skim milk, or synthetic diet 191 with 18 per cent casein. This observation is particularly striking in view of the fact that the protein content of the dried skim milk used was only 36.8 per cent. It must be pointed out, however, that other evidence indicates that mice on low protein regimens are particularly sensitive to the infection-enhancing effect of certain other components of the diet. This is apparent in the low resistance to infection of mice fed diets containing either 4 or 5 per cent skim milk, in which peanut oil had been replaced by cerelose (see Tables II, III, IV and Fig. 2). A similar deleterious effect was observed when sodium citrate or large amounts of cocoa butter were added to diets low in protein.

Comparative Effects of Cerelose and Various Fats in the Diets:

There have been many conflicting reports concerning the role of dietary fats in experimental tuberculosis (17, 18). Experimentation *in vitro* also yields a confusing picture of the effects of lipids on tubercle bacilli, the results ranging from marked enhancement to complete inhibition of growth depending upon the conditions under which the culture tests were carried out (19 to 21). In the course of the present study many experiments have been carried out to determine the effect on mouse tuberculosis of addition to the diet of various glycerides or fatty acids. Although the results do not yet appear in a clear pattern, they leave no doubt that the effect of dietary fats on infection is determined to a very large extent by the presence or absence of other substances in the diet. It is very likely furthermore that many of the effects observed are not caused directly by the fats themselves, but rather indirectly through the products of their intermediary metabolism. Out of the welter of results yielded by infection tests carried out over several years under a wide range of conditions, a few will be selected to illustrate the effect exerted on mouse tuberculosis by the replacement of cerelose by equal weights of plant or animal fats.

The percentage composition of some of the diets used is presented in Table III (three consecutive experiments). The fat (or cerelose) was mixed thoroughly by hand with the skim milk and wheat flour. Each batch of food was resuspended in an equal weight of 7.5 per cent gelatin in water. Food and drinking water were supplied *ad lib.* All animals were infected with 0.01 ml. of culture MV the day that they were placed on the experimental diets. The time of death of infected animals and the average weight gains of uninfected controls (over a total period of 3 weeks) are recorded in Table III.

The results presented in Table III do not reveal any obvious effect of the replacement of cerelose by either peanut oil, cocoa butter, or lard in diets containing 15 or 20 per cent skim milk. It is clear, however, that mice proved particularly susceptible to tuberculous infection when they were fed diets containing only 4 or 5 per cent skim milk, with cerelose but no added fat beyond that which was present in the skim milk and wheat flour. This infection-enhancing effect could be counteracted by adding peanut oil to the low skim milk diet, but not so well with cocoa butter. Table IV and Fig. 2 present the results of three consecutive experiments which illustrate the comparative effects of equal weights of cerelose or of peanut oil, added to diets containing only 4 or 5 per cent skim milk. The protective effect of peanut oil under these particular conditions is obvious.

Effect of Addition of Sodium Citrate to the Diet:

Two independent reasons prompted a study of the effect of adding sodium citrate to the diet on the course of tuberculosis. (*a*) On the one hand, it is

known that citric acid enhances the growth of tubercle bacilli *in vitro*, and can furthermore protect them against the toxic effects of lactic acid at slightly acidic reactions (13, 14). It seemed likely that these effects would also obtain *in vivo* and that addition of citrate to the diet might in consequence accelerate the course of tuberculosis. (*b*) On the other hand, it appeared probable that the presence *in vivo* of high concentrations of citric acid can modify some of the reactions of the Krebs cycle in the tissues, and thereby affect the accumulation of some metabolites associated with this cycle. Some of these

TABLE III

Effect on Mouse Tuberculosis of Addition to Diets of Equal Weights of Cerelose or Various Fats

Percentage composition of diets*						Weight gain	Cumulative number of mice (out of 10) dead at			
Skim milk	Wheat flour	Cerelose	Peanut oil	Cocoa butter	Lard		5 wks.	7 wks.	9 wks.	11 wks.
						gm.				
4	45	50	—	—	—	3.0	7	10		
4	45	30	20	—	—	3.8	1	2	3	—‡
4	45	30	—	20	—	3.1	3	4	6	—‡
5	74	20	—	—	—	8.2	0	3	7	10
5	74	—	20	—	—	11.0	0	1	7	8
5	74	—	—	20	—	8.9	0	5	8	10
15	64	20	—	—	—	10.8	0	2	7	8
15	64	—	20	—	—	10.1	0	1	5	7
15	64	—	—	20	—	10.5	0	2	4	6
20	50	30	—	—	—	Not Weighed	0	2	5	9
20	50	10	—	20	—	Not Weighed	0	4	7	9
20	50	10	—	—	20	Not Weighed	0	4	7	9

* 1 per cent salt added.

‡ —, experiment discontinued.

metabolites have been found to favor the multiplication and survival of tubercle bacilli (13, 14). The effect on mouse tuberculosis of diets supplemented with citric acid is illustrated in Tables V, VI, and VII and Figs. 3 A, 3 B, and 4.

The results plotted in Figs. 3 A (four experiments) and 3 B (two experiments) were obtained with diets containing 4, 5, 15, or 19 per cent skim milk. Their composition is outlined in Tables V and VI. All food mixtures were incorporated in an equal volume of 7.5 per cent gelatin in tap water and fed *ad lib.* to mice which had been infected intravenously the same day with 0.01 ml. culture MV.

TABLE IV

(see Fig. 2)

Effect of Peanut Oil on Tuberculosis of Mice Fed Diets Containing 4 or 5 Per Cent Skim Milk

Fig. No.	Percentage composition of diets*				Weight gain	Cumulative number of mice (out of 10) dead at				
	Skim milk	Wheat flour	Cerelose	Peanut oil		3 wks.	5 wks.	7 wks.	9 wks.	11 wks.
					gm.					
2-ac	4	45	50	—	3.0	1	7	10		—‡
2-ap	4	45	30	20	3.8	0	1	2	3	—‡
2-bc	5	74	20	—	8.2	0	0	3	7	10
2-bp	5	74	—	20	11.0	0	0	1	7	8
2-cc	5	74	20	—	Not Weighed	3	9	10		
2-cp	5	74	—	20	Not Weighed	1	2	8	—‡	

* 1 per cent salt added.

‡ —, experiment discontinued.

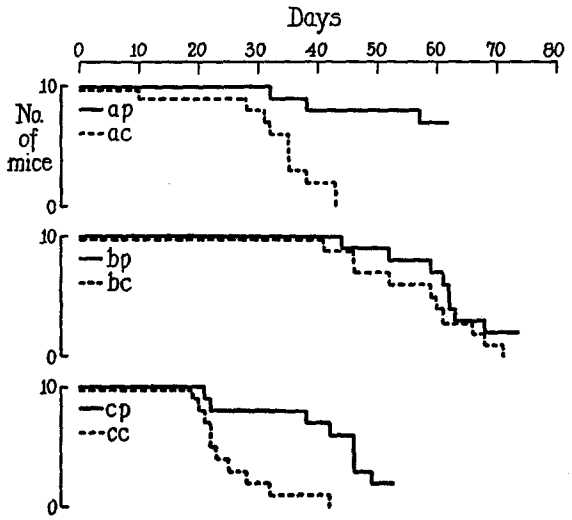

FIG. 2. Effect of peanut oil on tuberculosis of mice fed diets low in milk. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table IV.

TABLE V

(see Fig. 3 A)

Effect of Citrate in Diets Containing 4 to 5 per Cent Skim Milk

Fig. No.	Percentage composition of diets*						Weight gain	Cumulative number of mice (out of 10) dead at				
	Skim milk	Wheat flour	Cerelose	Peanut oil	Cocoa butter	Citrate		3 wks.	5 wks.	7 wks.	9 wks.	11 wks.
							gm.					
3 A-a	4	45	50	—	—	—	3.0	1	7	10		
3 A-ac	4	45	40	—	—	10	−0.5	3	8	9	10	
3 A-b	4	45	30	20	—	—	3.8	0	1	2	3	—‡
3 A-bc	4	45	20	20	—	10	0.4	0	9	10		
3 A-c	4	45	30		20	—	3.1	0	4	5	6	—‡
3 A-cc	4	45	20		20	10	0	3	10			
3 A-d	5	74	20	—	—	—	8.2	0	0	3	7	10
3 A-dc	5	66	20	—	—	8	2.5	0	0	6	10	
3 A-e	5	74	—	20	—	—	11.0	0	0	1	7	8
3 A-ec	5	66	—	20	—	8	6.7	0	0	3	10	
3 A-f	5	74	—	—	20	—	8.9	0	0	5	9	10
3-A-fc	5	66	—	—	20	8	5.8	0	1	9	10	
3 A-g	5	74	—	—	20	—	Not weighed	0	3	9	10	
3 A-gc	5	66	—	—	20	8	Not weighed	3	7	9	10	
3 A-h	5	74	—	20	—	—	Not weighed	1	2	8	10	
3-A-hc	5	66	—	20	—	8	Not weighed	2	6	9	10	

* 1 per cent salt added.

‡ — experiment discontinued.

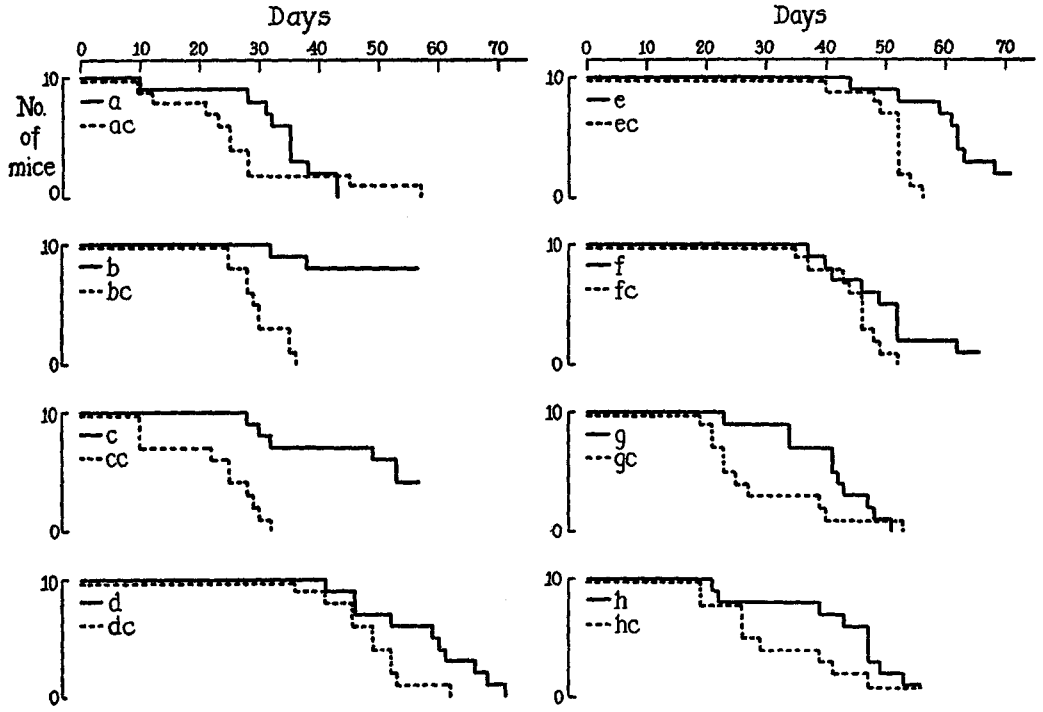

FIG. 3 A. Effect of citrate on mouse tuberculosis. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table V.

TABLE VI

(see Fig. 3 B)

Effect of Citrate in Diets Containing 15 to 19 per Cent Skim Milk

Fig. No.	Percentage composition of diets*							Weight gain	Cumulative number of mice (out of 10) dead at				
	Skim milk	Wheat flour	Cerelose	Peanut oil	Cocoa butter	Lard	Citrate		3 wks.	5 wks.	7 wks.	9 wks.	11 wks.
								gm.					
3 B-i	15	64	20	—	—	—	—	10.8	0	0	2	7	8
3 B-ic	15	56	20	—	—	—	8	6.1	1	1	4	10	
3 B-j	15	64	—	20	—	—	—	10.1	0	0	1	4	6
3 B-jc	15	56	—	20	—	—	8	7.3	0	1	3	8	9
3 B-k	15	64	—	—	20	—	—	10.5	0	0	2	4	6
3 B-kc	15	56	—	—	20	—	8	5.3	0	1	2	6	8
3 B-l	19	50	30	—	—	—	—	Not weighed	0	0	2	5	9
3 B-lc	19	50	20	—	—	—	10	Not weighed	1	3	5	8	10
3 B-m	19	50	10	—	20	—	—	Not weighed	0	0	4	7	9
3 B-mc	19	50	—	—	20	—	10	Not weighed	0	1	6	10	
3 B-n	19	50	10	—	—	20	—	Not weighed	0	0	2	8	10
3 B-nc	19	50	—	—	—	20	10	Not weighed	0	2	6	10	

* 1 per cent salt added.

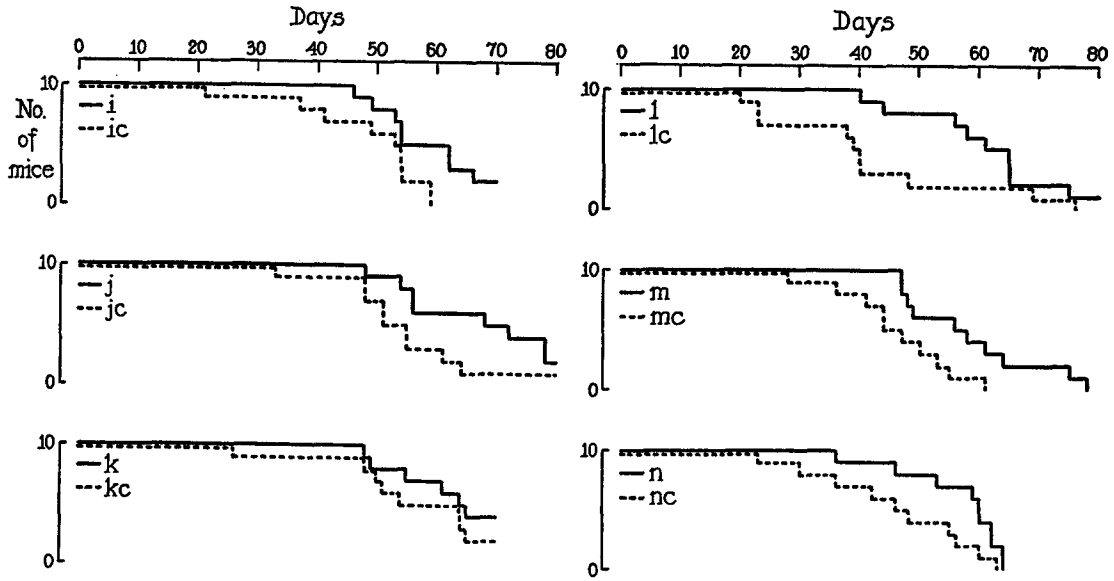

FIG. 3 B. Effect of citrate on mouse tuberculosis. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table VI.

Fig. 4 and Table VII present the results of an experiment in which all mice were fed dry pellets *ad lib.* and were given various solutions as drinking fluid (also *ad lib.*). Ten mice received 0.8 per cent NaCl; ten received a solution of 2 per cent citric acid adjusted to pH 6.0 with sodium hydroxyde; ten received a solution of 1 per cent glutaric acid also at pH 6.0; and ten, a solution of 5 per cent alcohol in 0.8 per cent sodium chloride.

The results presented in Tables V, VI, and VII and in Figs. 3 A, 3 B, and 4 show that addition of citric acid to any of the various diets tested shortened

TABLE VII

(see Fig. 4)

Effect on Mouse Tuberculosis of Citrate, Glutarate, or Alcohol Added to Drinking Fluid

Fig. No.	Diet	Drinking fluid	Cumulative number of mice (out of 10) dead at			
			3 wks.	4 wks.	5 wks.	6 wks.
4-a	Pellets *ad lib.*	0.8 per cent NaCl	0	1	5	10
4-b	" " "	2 per cent Na citrate (pH6.0)	2	7	9	10
4-c	" " "	1 per cent Na glutarate (pH6.0)	1	7	9	10
4-d	" " "	5 per cent alcohol in saline	0	2	7	9

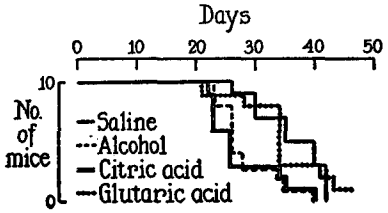

FIG. 4. Effect of citrate, glutarate, or alcohol on mouse tuberculosis. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table VII. The symbols for glutaric acid and alcohol have been inadvertently reversed in Fig. 4 (The data in Table VII are correct).

the expectancy of life of tuberculous mice. It will be noted in Table VII and Fig. 4 that 1 per cent glutarate administered in the drinking fluid enhanced infection as much as did citrate (only one experiment performed), whereas 5 per cent alcohol had no significant effect on susceptibility. In many tests to be reported in a subsequent paper, addition of lactic acid to the diet failed to enhance infection and indeed appeared to retard somewhat the death of tuberculous mice. Other experiments are under way to determine the effect of other organic acids on infection, as well as the minimum effective dose of citric acid. In two experiments, designed to test the latter point, no significant difference could be noted between the infection enhancing effects of 3, 5, 7, and 10 per cent citrate in the diet.

Acceleration of the course of experimental tuberculosis by citrate has been observed whether the mice were fed pellets, or diets containing 4, 5, 15, or 20 per cent skim milk, or 18 per cent casein. It must be pointed out, however, that the results with diets containing 30 or 33 per cent skim milk have been less consistent—as appears from the two experiments presented in Table VIII. Since the tests with high concentrations of skim milk have not yet been extended, it is not possible to discuss further the bearing of these early findings on the infection-enhancing effect of citrate.

Weight Gain and Susceptibility to Infection:

In order to determine whether there was any relation between growth curve and susceptibility to infection, groups of 6 to 10 mice on most of the diets

TABLE VIII

Effect of Citrate on Tuberculosis of Mice Fed Diets Containing 30 or 34 Per Cent Skim Milk

Percentage composition of diets*					Weight gain	Infective dose ml. MV	Cumulative number of mice (out of 10) dead at			
Skim milk	Wheat flour	Peanut oil	Cerelose	Citrate			3 wks.	5 wks.	7 wks.	9 wks.
					gm.					
30	60	—	9	—	Not weighed	0.1	7	10		
30	60	—	—	9	Not weighed	0.1	6	10		
30	60	—	9	—	Not weighed	0.01	0	0	5	6
30	60	—	—	9	Not weighed	0.01	0	0	5	6
34	65	—	—	—	8.8	0.01	0	0	4	4
34	15	20	20	10	0	0.01	0	0	1	5
34	15	—	40	10	−0.6	0.01	0	7	8	10

* 1 per cent NaCl added.

tested were kept as non-infected controls and weighed at weekly intervals. The average weight gains (per uninfected mouse during the initial period of 3 weeks on each regimen) are given in the tables or figures presenting the results of individual experiments.

Examination of weight gains in relation to the length of survival following infection reveals that all the regimens tested which proved capable of shortening the life expectancy of tuberculous mice also decreased the rate of growth of uninfected animals. It must be emphasized, however, that several regimens which caused marked retardation of growth failed to increase in a detectable manner the susceptibility of mice to tuberculosis. This was the case with chronic undernutrition brought about by maintaining the daily food intake to a constant level low enough to reduce drastically the weight gain of normal animals. Similarly, mice fed diets supplemented with peanut oil but low in

protein (4 or 5 per cent skim milk), gained weight only slowly (3.5 gm. over a period of 3 weeks) yet were fully as resistant to infection as mice which received *ad lib.* either pellets (25 per cent protein), or the synthetic diet 191 (18 per cent casein), or Sherman diet (33 per cent dried milk)—three high protein diets that allowed a much more rapid increase in weight. Furthermore, the mice on low protein–peanut oil diets were much more resistant than mice fed either pellets or diet 191, but deprived of food 30 consecutive hours every week, even though the two latter groups gained as much weight as did the

TABLE IX

(see Fig. 5)

Effect of Weight Gain of Mice on Resistance to Tuberculosis

Fig. No.	Percentage composition of diets*					Weight gain	Cumulative number of mice (out of 10) dead at		
	Skim milk	Wheat flour	Cerelose	Peanut oil	Citrate		3 wks.	5 wks.	7 wks.
						gm.			
5-a	4	45	30	20	—	3.8	0	1	2
5-b	4	45	50	—	—	3.0	1	7	10
5-c	4	45	40	—	10	0	3	8	10
5-d	34	65	—	—	—	8.8	0	0	4

* 1 per cent salt added.

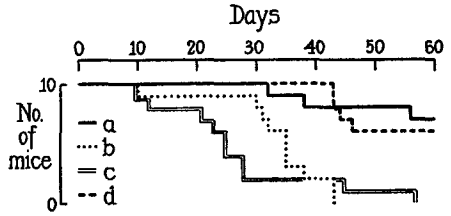

FIG. 5. Weight gain and resistance to tuberculosis in mice. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table VIII.

former. Many similar discrepancies which are revealed by a comparison of mortality curves among tuberculous mice and of weight curves among non-infected groups, make clear that the susceptibility to infection cannot be predicted from the effects exerted by the dietary regimens on the growth curve. This lack of correlation appears strikingly from the results of the experiment selected for illustration in Table IX and Fig. 5.

Effect of Dinitrophenol and Thyroid Extract:

It is known that dinitrophenol can interfere with the synthesis of glycogen by tissue cells and that prolonged excessive feeding of thyroid extract reduces

greatly the store of glycogen in the liver (22, 23).There is evidence on the other hand that leucocytes can convert this polysaccharide into lactic acid and use it as a source of energy in some phases of phagocytosis (24, 25). These facts suggested that treatment with dinitrophenol or thyroxine might affect unfavorably the resistance of mice to tuberculosis by depleting their glycogen reserves.

Preliminary experiments were conducted to determine the minimum quantity of dinitrophenol, or of thyroid extract, that had to be added to an otherwise normal diet to reduce by approximately two-thirds the rate of growth of normal mice. As shown in Table X, addition of 90 mg. of dinitrophenol to 100 gm. of a wheat flour–skim milk diet reduced drastically the weight gain of normal mice. The corresponding dose for the preparation of thyroid extract used was 200 mg. per 100 gm. of diet.

TABLE X

Effect of Dinitrophenol on Weight Curves of Uninfected Mice

Percentage composition of diet*			Weight‡ after following times on experimental diets				Total change at day 15
Skim milk	Wheat flour	Dinitrophenol	Initial	5 days	10 days	15 days	
			gm.	*gm.*	*gm.*	*gm.*	*gm.*
33	66	0.27	93	90	83	91	−2
33	66	0.09	89	93	93	97	+8
33	66	0.03	84	91	94	100	+16
33	66	0	84	92	97	104	+20

* 1 per cent salt added.

‡ total for four mice weighed together.

The effect of dinitrophenol and thyroid extract on mouse tuberculosis was determined in several experiments which are presented in Tables XI, XII, XIII, and XIV, and in Figs. 6, 7, 8, 9, and 10.

In the experiment corresponding to Table XI, and Figs. 6 and 7, seven groups of 10 mice each were infected with 0.01 ml. culture MV and fed pellets *ad lib.* One group of 10 mice received 0.8 per cent NaCl as drinking fluid (control); three groups were given solutions of dinitrophenol (in saline) in concentrations of 0.1, 0.05, or 0.025 per cent; the three other groups received solutions (also in saline) of thyroid extract in concentration of 0.03, 0.01, or 0.003 per cent. (Thyroid U.S.P. powder No. 58—Eli Lilly and Co.—stated to contain not less than 0.17 nor more than 0.23 per cent iodine.) All experimental regimens were started on the day of infection.

It is clear from the results presented in Table XI and Figs. 6 and 7 that infected mice receiving the lowest concentrations of dinitrophenol (0.025 per cent) or of thyroid extract (0.003 per cent) survived at least as long as those

receiving saline. An infection-enhancing effect was apparent only with the higher concentrations of these drugs.

In the experiment corresponding to Table XII and Fig. 8 half of the mice were infected intravenously with 0.004 ml. and the other half with 0.1 ml. of culture MV. Immediately after infection, they were placed, and maintained thereafter, on a diet consisting of wheat

TABLE XI

(see Figs. 6 and 7)

Effect of Dinitrophenol and Thyroid Extract on Mouse Tuberculosis

Fig. No.	Diet	Drinking fluid containing (*per cent*)		Cumulative number of mice (out of 10) dead at		
		Dinitrophenol	Thyroid extract	4 wks.	5 wks.	6 wks.
6-a, 7-a	Pellets *ad lib.*	0	0	1	5	10
6-b	" " "	0.0025	0	2	4	6
6-c	" " "	0.005	0	4	7	10
6-d	" " "	0.01	0	4	7	10
7-b	" " "	0	0.003	2	3	7
7-c	" " "	0	0.01	4	8	10
7-d	" " "	0	0.03	10	—	—

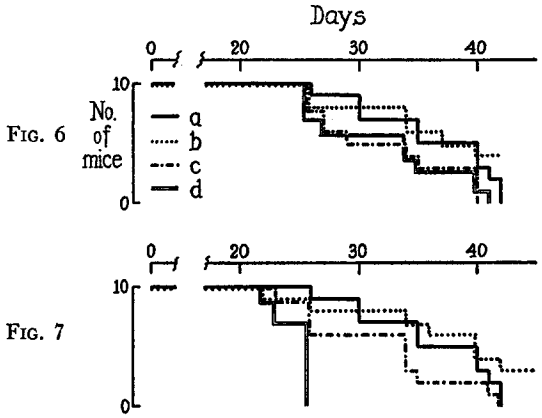

FIG. 6

FIG. 7

FIGS. 6 and 7. Effect of dinitrophenol and thyroid extract on mouse tuberculosis. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table XI.

flour (66), skim milk (33), and salt (1)—with or without dinitrophenol (100 mg. per 100 gm. diet). The diets were resuspended in an equal weight of 7.5 per cent gelatin in tap water and fed *ad lib.* The results are presented in curves A_1, D_1, A_2, D_2 of Fig. 8.

In similar experiments, dinitrophenol in concentration of 0.1 per cent of the food (dry weight) was added to either the skim milk–white flour diet, or to synthetic diet 191. All animals were infected with 0.01 ml. MV and placed on the experimental diets on the day of infection or 2 days later (curves A, AD, B, and BD of Fig. 9, Table XIII).

As can be seen from the results presented in Tables XI, XII, XIII, and Figs. 6, 7, 8, and 9 (curves A, AD, B, and BD) the infection-enhancing effect of dinitrophenol, or of thyroid extract, was apparent only in the mice that died approximately 4 weeks after infection or later. No effect could be detected when a large infective dose was used (0.1 ml. of culture MV), most of the animals in this case dying within 24 days after infection. Another experiment was therefore designed to test whether the effect of dinitrophenol could be made evident earlier by using a smaller infective dose and beginning administration of the drug a few days before infection.

TABLE XII

(see Fig. 8)

Effect of Infective Dose on Enhancement of Mouse Tuberculosis by Dinitrophenol

Fig. No.	Percentage composition of diet*			Infective dose (ml. MV)	Cumulative number (out of 10) of mice dead at			
	Skim milk	Wheat flour	Dinitrophenol		3 wks.	5 wks.	7 wks.	9 wks.
8-A1	33	66	—	0.1	7	9	10	10
8-D1	33	66	0.1	0.1	8	10	10	10
8-A2	33	66	—	0.004	0	0	5	7
8-D2	33	66	0.1	0.004	0	5	8	10

* 1 per cent salt added.

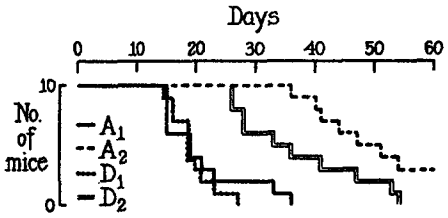

FIG. 8. Effect of dinitrophenol on mouse tuberculosis. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table XII.

In the experiment corresponding to curves C and CD of Fig. 9 (Table XIII), the mice were infected with a very small dose (0.001 ml. of culture MV). They were fed a diet consisting of wheat flour (66), skim milk (33), and salt (1) with or without dinitrophenol (100 mg. per 100 gm., of diet); these food mixtures were incorporated in an equal weight of 7.5 per cent gelatin in tap water and fed *ad lib.* The animals were placed on the experimental diets 5 days before infection.

The infection-enhancing effect of dinitrophenol illustrated in curves C and CD of Fig. 9 became apparent on the 18th day after infection, approximately 1 week earlier than had been observed in the preceding tests. Taken together, the results presented in Figs. 6, 7, 8, and 9 suggest therefore that the effects

exerted by dinitrophenol and thyroid extract on infection are not immediate, but have a latent period of at least several days. In only one case was it pos-

TABLE XIII

(see Fig. 9)

Effect of Time of Administration of Dinitrophenol on Mouse Tuberculosis

Fig. No.	Percentage composition of diet*			Infective dose (ml. MV)	Diet started at following time	Cumulative number of mice (out of 10) dead at			
	Skim milk	Wheat flour	Dinitrophenol			3 wks.	5 wks.	7 wks.	9 wks.
9-A	33	66	—	0.01	2 days after infection	3	3	4	—‡
9-AD	33	66	0.1	0.01		3	7	10	—‡
9-B	(Diet 191)		—	0.01	Same day as infection	0	0	4	6
9-BD	(Diet 191)		0.1	0.01		0	0	9	10
9-C	33	66	—	0.001	5 days before infection	0	1	1	—‡
9-CD	33	66	0.1	0.001		3	7	10	—‡

* 1 per cent salt added.

‡ —, experiment discontinued.

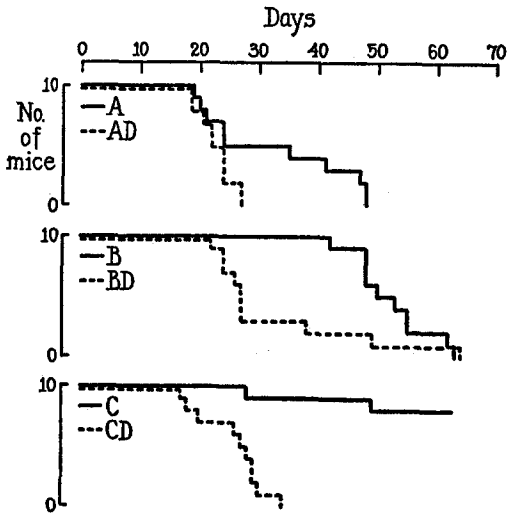

FIG. 9. Effect of dinitrophenol on mouse tuberculosis. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table XIII.

sible to detect a very early effect of dinitrophenol, namely in mice which had been previously vaccinated with BCG.

Three groups of ten mice each were vaccinated intraperitoneally with 0.02 ml. of culture BCG-P grown in liquid tween-medium containing 0.1 per cent human albumin. They were

challenged 3 weeks later by the intravenous injection of 0.2 ml. of culture MV, grown in bovine albumin. On the same day, they were placed on experimental diets (*ad lib.*) consisting of wheat flour (66), skim milk (33), salt (1) with or without thyroid extract or dinitrophenol in a final concentration of 80 mg. per 100 gm. of dry food. The results are presented in Table XIV and Fig. 10.

Here again, treatment with thyroid extract and dinitrophenol shortened the life expectancy of the tuberculous mice; the results with thyroid extract were less striking than in the experiments presented in Fig. 7, perhaps because the concentration of hormone used was much smaller. In contrast with what had been observed in preceding experiments (Figs. 6, 8, and 9), the effect of

TABLE XIV

(see Fig. 10)

Effect of Dinitrophenol and Thyroid on Tuberculosis in Vaccinated Mice

Fig. No.	Percentage composition of diets*				Vaccination (0.2 ml.)	Infection (0.2 ml. MV)	Cumulative number of mice (out of 10) dead at			
	Skim milk	Wheat flour	Dinitro-phenol	Thyroid extract			2 wks.	3 wks.	4 wks.	5 wks.
11-A	33	66	—	—	BCG	3 wks. after	0	3	4	8
11-D	33	66	0.08	—	BCG	vaccina-	4	5	7	10
11-T	33	66	—	0.08	BCG	tion	0	7	10	10

* 1 per cent salt added.

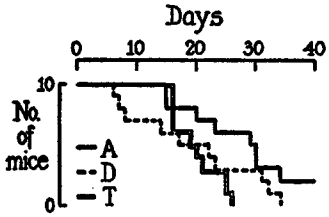

FIG. 10. Effect of dinitrophenol on tuberculosis of vaccinated mice. Ordinates indicate number of mice (out of 10) surviving at different periods of time after infection (abscissae). For experimental details, see Table XIV.

dinitrophenol became apparent very soon after infection. However, the significance of this observation is obscure since the mice certainly harbored large numbers of BCG organisms in their organs at the time that they were placed on the experimental diets. It is possible that the physiological effects associated with BCG infection had rendered the vaccinated animals more susceptible to dinitrophenol. Moreover, as will be presently shown, administration of this drug allows BCG to establish a fatal infection in mice.

Effect of Metabolic Disturbances on the Susceptibility of Mice to BCG:

All the infection-enhancing effects which have been described in the preceding parts of this paper were observed by using a mouse virulent myco-

bacterium (MV) for the infection tests. Many similar experiments have been carried out with attenuated cultures of mammalian tubercle bacilli (R1Rv and several substrains of BCG) as well as with staphylococci. Detailed description of the findings with these microorganisms will be presented in subsequent reports (26, 27). Suffice it to outline here the results obtained with the culture BCG-P.

Mice were infected with 0.2 ml. of 10 day old culture BCG-P in tween-albumin medium and immediately placed on experimental diets containing citrate or dinitrophenol. The composition of the diets and concentrations of the additives are indicated in Table XV.

The results presented in Table XV make it clear that most of the mice infected with BCG-P developed a slowly progressive fatal disease when fed

TABLE XV

*Effect of Addition to Diet of Citrate or Dinitrophenol on Susceptibility of Mice to BCG**

Percentage composition of diets‡					Dinitrophenol	Cumulative number of mice (out of 10) dead at				
Skim milk	Wheat flour	Peanut oil	Cerelose	Citrate		5 wks.	7 wks.	9 wks.	11 wks.	13 wks.
20	50	20	10	—	—	0	0	0	1	1
20	50	20	—	10	—	2	3	4	7	8
20	50	20	10	—	0.1	1	3	5	6	7

* 0.2 ml BCG-P injected intravenously.

‡ 1 per cent salt added.

diets containing citrate or dinitrophenol, substances which have been shown to accelerate the course of virulent tuberculous infection.

DISCUSSION

The findings described in the present report leave no doubt that it is possible to decrease at will the resistance of albino mice to tuberculous infection by simple manipulations of the dietary regimens. However, the effects obtained do not appear to have been the direct result of nutritional deficiencies *per se*, but rather the indirect expression of metabolic disturbances set in motion by the various experimental procedures.

The lack of correlation between enhancement of infection and effect of the dietary regimen on the weight curves of the uninfected animals was to be expected. It had already been found by other investigators that mice fed a diet enriched with a variety of natural food products (cereals, yeast, cod liver oil, fish, meat, and bone meat, etc.) showed higher resistance to tuberculosis than mice receiving Sherman diet, although they grew approximately as well on the latter as on the former regimen. Of six natural diets fed mice infected

with *Salmonella* or tubercle bacilli "the best diet for reproduction, which was also a good diet for growth, was the poorest for resistance to infection" (4, 5, 11). In the present study, it was found that mice gained weight on some of the citrate-containing diets which caused them to die rapidly of tuberculosis. In contrast, the disease was little if at all aggravated in animals which were prevented from gaining weight by being maintained in a state of chronic undernutrition by limiting to a low, but constant level their total food intake or the protein content of their diet. On the other hand, animals completely deprived of food for 30 consecutive hours every week, and fed optimum diets *ad lib.* at other times, did gain weight but became highly susceptible to infection. Recent experiments with staphylococci and Friedländer bacilli have revealed that the resistance of fasted animals to infection returns to normal within a short time after feeding has been resumed (28). The contrast between chronic undernutrition and acute starvation with regard to infectious agents had already been observed in rats and mice infected with *Salmonella typhimurium*. Whereas progressive reduction of the food intake during this experimental disease stimulated the rate of bacterial multiplication *in vivo*, restriction down to 80 per cent of the optimum amount proved to have no influence provided the restricted regimen was maintained at a constant level from day to day (29).

In many tests not described in this report, the resistance of mice to infection appeared to be unaffected by addition to the diet of any of the vitamins (including vitamin B12 and large amounts of fat-soluble vitamins). This finding may be of little significance since the diets used contained wheat flour and skim milk which probably provided a fairly adequate vitamin complement. More surprising was the failure to observe any deleterious effect of protein limitation. The level of resistance observed with some of the diets containing only 4 per cent skim milk was as high as that observed with any of the other experimental regimens tested, however high their protein content. Thus, tuberculous mice died at the same rate whether they received high protein diets (pellets containing 25 per cent of mixed protein; synthetic diet 191 with 18 per cent casein; wheat flour supplemented with 15, 20, or 33 per cent skim milk) or a diet containing only 7 per cent organic nitrogen (1.5 per cent supplied in the form of 4 per cent skim milk, and 5.5 per cent supplied by the wheat flour). It must be emphasized however that susceptibility to infection on this low protein diet was exceedingly high when the caloric balance was made up exclusively with cerelose. Resistance became normal only when part of the cerelose was replaced by peanut oil, or some other fat.

Despite common belief, published information does not provide a clear picture of the role of protein intake on resistance to infectious diseases. In general, manipulation of the protein level in the diet has been found to have but little effect on the outcome of experimental infections, unless depletion

was so complete and prolonged as to impair profoundly the general health of the animal. The results obtained in experimental tuberculosis of the rat, guinea pig, and hamster have been contradictory and provide little support for the view that the level of dietary protein plays an important part in resistance to this disease (1, 4–9, 11, 29–33).

Whereas the experiments reported in the present paper provided no indication that protein deficiency *alone* could accelerate the course of tuberculosis there was some evidence that mice fed diets containing only small amounts of skim milk became more sensitive to certain infection-enhancing effects resulting for example from excessive intake of cerelose, cocoa butter, or citrate. In man, the circumstances (individual or epidemiological) which lead to a decrease in protein consumption are usually conducive to other simultaneous nutritional aberrations. It may be worth noting in this respect that investigators who have tried to correlate deficiency in nutritional factors with susceptibility to tuberculosis on the basis of epidemiological studies of the human disease have emphasized the importance of proteins of *animal source.* As deficiency in foods of animal origin is almost always accompanied by other dietary limitations, it seems possible that these, rather than low protein intake, are responsible for the increase in susceptibility to tuberculosis which has been observed in certain epidemiological situations, for example during war time.

As mentioned in the introduction, the design of many of the experiments reported in the present paper was based on the working hypothesis that resistance to infection is conditioned by the relative concentration in the infected tissues of lactic acid on the one hand, and of certain polycarboxylic acids and acetone bodies on the other (12–15). It need not be pointed out that, because of their essentially biological nature, the observations reported here do not provide evidence for or against this view. The most that can be said is that the results obtained were compatible with some of the consequences to be expected from the working hypothesis.

It is known for example that in man and large animal species, ketosis is present during the early phase of acute fasting, but is usually absent in chronic undernutrition. Since keto acids favor the multiplication of tubercle bacilli under certain circumstances, it appears possible that the ketosis which occurs during the early period of acute fasting may cause a temporary stimulation of bacillary metabolism. Experiments in progress indicate that mouse tuberculosis can be rendered more severe by the addition to the diet of certain fatty acids with short carbon chains, the oxidation of which is known to lead to the formation of acetone bodies *in vivo.*

Whatever the diet used, addition to it of sodium citrate reduced consistently the life expectancy of mice infected with tubercle bacilli. Definite effects were obtained with daily intakes of 0.1 gm. citrate per mouse. Addition

of alcohol or sodium lactate to the diet even in large amounts had no apparent effect on the course of the disease. Worth mentioning here is the fact that daily injections of citric acid into mice has been shown by others to increase the susceptibility of these animals to infection with *Salmonella typhimurium* (34, 35).

Two hypotheses can be considered to explain the enhancement of tuberculosis by citrate. On the one hand, citric acid is known to favor the multiplication of tubercle bacilli *in vitro*, and it may be surmised that it favors also their multiplication *in vivo*. In addition to this direct effect on the bacilli, citric acid may influence the course of infection in an indirect manner by modifying tissue metabolism, perhaps at some stage in the Krebs cycle. It has been found in fact that addition of citrate to many of the experimental diets reduced markedly the rate at which uninfected animals gained weight. Available information does not seem to warrant any further discussion of the actual mechanism by which citrate aggravated the course of tuberculosis in the present experiments.

One of the first consequences of acute fasting is the exhaustion of the glycogen reserves of the body. Since phagocytic cells utilize glycogen as source of energy (24, 25), their behavior is most probably altered during certain phases of fasting. It can also be presumed that glycogen depletion will bring about a decrease in lactic acid production by tissue cells and thus jeopardize one of the normal tissue reactions in the inflammatory areas (12–15). A similar effect may follow prolonged treatment with dinitrophenol and thyroid extract, since these substances interfere with the accumulation of glycogen by decreasing the efficiency with which phosphorylations are coupled to oxidations (22, 23).

Any attempt to explain the mechanisms by which thyroxine and dinitrophenol accelerate the course of mouse tuberculosis brings into sharp focus the superficiality of the present discussion. It is obvious that the administration of either of these two metabolic stimulants sets in motion a large number of complex processes with a multiplicity of ill defined secondary effects. Similarly, any modification of the nutritional status has repercussions far removed from the initial metabolic disturbance that it causes—involving in many cases various components of the hormonal system. Stimulation of the adrenal cortex for example, might account for many of the phenomena observed, by bringing about an overproduction of cortisone. (Let it be mentioned in passing that cortisone, by reducing the inflammatory reaction, most likely decreases the production of lactic acid at the site of the lesion). Many other mechanisms should be considered as susceptible of playing some role in the decrease in resistance to infection which has been observed in the present experiments. Thus, it is possible that, instead of providing a more favorable environment for the multiplication of tubercle bacilli, the procedures used merely served

to render the animal tissues more susceptible to the bacterial toxins. It is clear that mere observation of survival time and weight curves cannot provide an answer to these questions. New techniques will be required to analyze in other terms the complex relationships between host and parasite under various metabolic conditions.

Irrespective of their mechanism, the infection-enhancing effects observed in the present study may contribute to the understanding of certain problems of the pathogenesis of infection. It has been found that the resistance of mice to tuberculosis can be markedly decreased by metabolic disturbances of short duration (for example as caused by the removal of food for 30 hours); a similar effect obtains in the case of infections with Friedländer bacilli and staphylococci as will be shown in other publications (27, 28). The fact that a *transient* physiological upset can increase so markedly the receptivity to infection bids fair to throw light on some obscure epidemiological situations. It has been shown furthermore, and will be illustrated more extensively elsewhere (26), that the procedures which increase susceptibility to virulent tubercle bacilli can also break down the resistance which mice normally display against attenuated organisms like BCG. This fact gives support to the belief that the physicochemical environments provided by the phagocytic cells and by the inflammatory response, will prove to be decisive factors in determining the ability of mycobacteria to behave as pathogenic agents.

SUMMARY

Mice maintained on various types of diets were found to become more susceptible to tuberculosis when deprived of food for periods of 30 hours shortly after infection. In contrast, the susceptibility of the animals to the disease was unaffected by undernutrition resulting from limitation of food intake to a low but constant daily level.

The resistance of mice to tuberculosis appeared to be independent—within wide limits—of the protein content of the diet. It is true that mice fed a diet very low in protein and high in carbohydrate proved highly susceptible, but resistance was normal if part of the carbohydrate was replaced by fat (peanut oil)—without any change in the protein content of the food.

Resistance to tuberculosis could be consistently and markedly decreased by adding sodium citrate (or glutarate) to a variety of diets.

The survival time following infection was greatly shortened if dinitrophenol or thyroxine were administered *per os* in amounts sufficient to limit the weight gains of non-infected controls. There was usually a lag period of several days before the infection-enhancing effect of these metabolic stimulants became manifest.

The procedures which increased the susceptibility of mice to infection with

virulent tubercle bacilli also made it possible to establish in these animals a fatal infection with BCG.

There was no constant relation between weight gains of uninfected mice on the various regimens, and the effect of the latter on susceptibility to tuberculosis.

These findings appear compatible with, but do not prove, the hypothesis that a decrease in resistance to infection can be brought about by metabolic disturbances which cause either a depletion of the glycogen reserves of the body, or a reduction in the glycolytic activity of inflammatory cells, or an increase in the concentration of certain polycarboxylic acids and ketones in the tissues.

BIBLIOGRAPHY

1. *Nutrition Rev.*, 1952, **10,** 141.
2. Clausen, S. W., *Physiol. Rev.*, 1934, **14,** 309.
3. Dubos, R. J., and Pierce, C. P., *Am. Rev. Tuberc.*, 1948, **57,** 287.
4. Howie, J. W., *Brit. J. Nutrition*, 1949, **2,** 331.
5. Howie, J. W., and Porter, G., *Brit. J. Nutrition*, 1950, **4,** 175.
6. Koerner, T. A., Getz, H. R., and Long, E. R., *Proc. Soc. Exp. Biol. and Med.*, 1949, **71,** 154.
7. Metcoff, J., *Am. J. Pub. Health*, 1949, **39,** 862.
8. Ratcliffe, H. L., *Fed. Proc.*, 1951, **10,** 368.
9. Ratcliffe, H. L., *Fed. Proc.*, 1954, **13,** (pt. 1), 441.
10. Robertson, E. C., *Medicine*, 1934, **13,** 123.
11. Sengupta, S. R., and Howie, J. W., *Brit. J. Nutrition*, 1949, **2,** 313.
12. Dubos, R. J., *J. Exp. Med.*, 1950, **92,** 319.
13. Dubos, R. J., *J. Exp. Med.*, 1953, **97,** 357.
14. Dubos, R. J., *J. Exp. Med.*, 1953, **98,** 145.
15. Dubos, R. J., Biochemical Determinants of Microbial Diseases, Cambridge, Harvard University Press, 1954.
16. Schneider, H. A., and Webster, L. T., *J. Exp. Med.*, 1945, **81,** 359.
17. Cirio, L., and Rosso, C., *Pathologica*, 1947, **39,** 79, quoted in *Chem. Abstr.* 1947, **41,** 5198.
18. Hedgecock, L. W., *Proc. Soc. Exp. Biol. and Med.*, 1948, **68,** 106.
19. Dubos, R. J., *J. Exper. Med.*, 1947, **85,** 9.
20. Nieman, C., *Bact. Rev.*, 1954, **18,** 147.
21. Patnode, R. A., *Am. Rev. Tuberc.*, 1954, **69,** 710.
22. Simon, E. W., *Biol. Rev.*, 1953, **28,** 453.
23. Barker, S. B., *Physiol. Rev.*, 1951, **31,** 205.
24. Wagner, R., and Jourke, A., *Arch. Biochem.*, 1952, **39,** 174.
25. Bazin, S., Delaunay, A., and Avice, C., *Ann. Inst. Past.*, 1953, **85,** 774.
26. Pierce, C. H., and Dubos, R. J., data to be published.
27. Smith, I., and Dubos, R. J., data to be published.
28. Dubos, R. J., Smith, I., and Schaedler R., data to be published.

29. Guggenheim, K., and Buechler, E., *J. Hyg.*, 1947, **45,** 103.
30. Cannon, P. R., *Nutrition Rev.*, 1949, **7,** 161.
31. Miles, J. A. R., *Brit. J. Exp. Path.*, 1951, **32,** 285.
32. Schneider, H. A., *Vitamins and Hormones*, 1946, 35.
33. Schneider, H. A., Nutrition Fronts in Public Health, New York, The National Vitamin Foundation, 1951, Series No. 3.
34. Berry, L. J., and Mitchell, R. B., *J. Infect. Dis.*, 1953, **93,** 75.
35. Berry, L. J., Merrit, P., and Mitchell, R. B., *J. Infect. Dis.*, 1954, **94,** 144.