STUDIES ON THE VIRULENCE OF TUBERCLE BACILLI. THE EFFECT OF CORD FACTOR ON MURINE TUBERCULOSIS.

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THE term " cord factor " has been previously suggested to designate a bacterial constituent extracted from cultures of tubercle bacilli characterised by the formation of " cords " under proper conditions *in vitro* (Bloch, 1950*a*). This growth pattern is typical of virulent and certain attenuated strains of *Mycobacterium tuberculosis* (Middlebrook, Dubos and Pierce, 1947). The chemistry of cord factor has been extensively studied (Bloch, Sorkin and Erlenmeyer, 1953 ; Noll and Bloch, 1953 ; Asselineau, Bloch and Lederer, 1953). It is an ester of mycolic acid, glucose and a small nitrogen-containing fragment (Noll, 1953 ; Noll and Bloch, 1955). Uniform preparations of a high degree of purity have been obtained from a number of virulent human and bovine strains of tubercle bacilli. The preparations used in the present study were extracted from various batches of the human strains H37Rv and " Brévanne." Their chemical identity and purity have been ascertained by combustion analysis, infra-red spectrum and optical rotation. These values are highly characteristic (Noll and Bloch, 1955).

The present study attempts to find direct evidence for the rôle played by cord factor in the course of a tuberculous infection. It was originally suggested that this bacterial component was concerned in the virulence of the tubercle bacillus (Bloch, 1950*a*). Subsequently, a number of observations and experimental findings have been published in support of this hypothesis (Bloch, 1950*b* ; Bloch and Noll, 1953*a*, *b*). The evidence was circumstantial rather than direct. It was based on suggestive correlations between the amounts of cord factor produced by tubercle bacilli and their virulence under various experimental conditions. This paper describes the direct influence of cord factor injections on experimental tuberculosis, with particular emphasis on the specificity of the observed effects.

MATERIAL AND METHODS.

Cord factor.—The material used had the following chemical specifications. Melting point : 38–42°. Acidity : 1·0 mm.^{3} 0·100 N–CH_3ONa/mg. Optical rotation : $[\alpha]_D^{25}$ 29–30° (c = 0·9, in benzene). Elementary analysis : C 76·5 per cent, H 12·5 per cent, N 0·9 per cent. Infra red spectrum : absorption maxima at : 3350, 2900, 1715, 1475, 1380, 1275, 1170, 1150, 1100, 1075, 1050, 1020, 995, 940, 915, 805, 720 cm.$^{-1}$.

Animals.—Two strains of mice were used : CF1 albinos, from Carworth Farms, New City, N.Y., and C57 Blacks, from Jackson Memorial Laboratories, Bar Harbor, Me. All were males, weighing 15–20 g. They were fed a standard diet of pellets (Rockland Mouse Diet) and water, and caged in groups of not more than 10 per cage. The rabbits were light-skinned males of a mixed breed, from Carworth Farms.

Mode of infection.—The mice were infected intravenously with a standard strain of tubercle bacilli (H37Rv) obtained from The Trudeau Laboratory, Saranac Lake, N.Y. The bacteria

were propagated and injected in liquid Tween-albumin medium (Dubos and Middlebrook, 1947), unless stated otherwise. Strains of *Staphylococcus aureus*, *Streptococcus pneumoniae* Type III, and haemolytic streptococci were received through the courtesy of the Department of Bacteriology at New York University. They were kept in the dry-frozen state and propagated before use in beef-heart broth containing about 5 per cent horse blood.

EXPERIMENTAL.

Toxicity of Cord Factor for Mice.

It has been previously stated that in order to show its characteristic toxicity for mice, cord factor has to be injected repeatedly and at intervals (Bloch, 1950*a*). This fact has since been confirmed in numerous tests and the technique described in detail (Bloch *et al.*, 1953 ; Bloch and Noll, 1953*a*). With the preparations used in the present experiments, 5 injections of 5 μg. each, suspended in 0·05 to 0·2 ml. light mineral oil (Bayol F) or olive oil and spaced 2 to 3 days apart from one another, killed at least 50 per cent of the mice between 7 and 28 days following the first injection, whereas a single injection of up to 1 mg. failed to kill. Typical data of such a test have been published (Bloch *et al.*, 1953). Each injection was followed by a severe drop in weight and, as a rule, the spacing of the repeated injections was guided by the weight changes of the mice, subsequent injections being given when the animals had regained their weight before the previous injection. The significance of the time interval was not known.

TABLE I.—*Effect of Five Injections of* 0·01 *mg. Cord Factor, given at Different Time Intervals.*

Intervals between injections.	Individual survival times (days after the first injection) (Ten C57 Black mice per group).
1 day	4, 8, 8, 8, 10, 11, 11, 11, 19, 21
2 days	8, 8, 9, 9, 9, 11, 14, 16, 17, 18
4 ,, ...	12, 13, 15, 16, 16, 20, 22, 39, 42, S*
8 ,, ...	17, 25, 35, 38, 43, S, S, S, S, S
14 ,, ...	S, S, S, S, S, S, S, S, S, S

* S indicates mouse was sacrificed 80 days after the first injection, when the experiment was discontinued.

In the following experiment (Table I), groups of C57 Black mice received equal doses of cord factor, but the injections were given at different time intervals. Every group was injected 5 times with 0·01 mg. cord factor. Table I shows that there was no significant difference between the groups injected daily, every other day and every 4th day. When the injections were spaced 8 days apart, the number of mice dying from these injections decreased markedly, and even more so when 2 weeks were allowed to elapse between injections.

When a preparation of cord factor was tested in progressively decreasing amounts, a clear end point was reached beyond which toxicity was no longer detectable, with either one or repeated injections. Chemically pure preparations were toxic at a minimal dose of 2·5–5 μg. per mouse, injected 5 times successively. However, with doses larger than the minimum, repeated injections were not correspondingly more toxic ; *e.g.*, the results of 5 injections of 0·005 mg. of a pure preparation did not differ significantly from the effects of 5 injections of 0·1 mg. of the same material.

The peculiar circumstances in which cord factor was toxic and the gross pathological lesions it produced, were reminiscent to some extent of the Shwartzman phenomenon. Therefore, an experimental attempt was made to determine whether cord factor gave a local or generalised Shwartzman phenomenon. All results were negative. In the rabbit no local or generalised Shwartzman phenomenon could be elicited by two cord factor injections. Likewise, cord factor was ineffective in any combination with meningococcus toxin or with starch. In the mouse, it was not possible to influence the toxicity of cord factor by various doses of nitrogen mustard or thorotrast, substances which affect the outcome of the generalised Shwartzman phenomenon in the rabbit (Stetson and Good, 1951; Good and Thomas, 1952).

Equally unsuccessful were attempts to modify the toxicity of cord factor in the mouse by the following antibiotics and drugs: streptomycin, para-aminosalicylic acid, isoniazid, thiosemicarbazone (TBI), penicillin, Triton A 20 and four other related surface-active agents found to have a therapeutic effect in murine tuberculosis by Cornforth, Hart, Rees and Stock (1951). Rutin (" Vitamin P "), which is thought to reduce capillary permeability, and pyribenzamine likewise had no effect.

Hence, neither by direct nor indirect methods has it been possible to relate the toxicity of cord factor to the type of tissue reaction known as the Shwartzman phenomenon. The ineffectiveness of pyribenzamine seems to indicate that the delayed toxicity is not related to allergic types of reactions, although more extensive experimentation is needed to preclude this possibility. Antituberculous drugs were likewise ineffective in modifying the toxicity of repeated injections of cord factor.

Effect of Cord Factor Injections on Experimental Tuberculosis.

If cord factor is concerned with the virulence of the tubercle bacillus, a combination of cord factor injections and bacterial infection should result in enhancement of the effects of infection. This has been shown to be the case in three types of experiment of which representative examples are illustrated in Tables II and III.

Table II shows an experiment where mice were injected intraperitoneally with one dose of cord factor in Bayol F followed 24 hr. later by an intravenous injection of various dilutions of a 5-day Tween-albumin culture of tubercle bacilli. There were 10 mice per group and 2 sets of infection controls, one receiving an intraperitoneal injection of Bayol F alone 24 hr. before infection, the other no injection prior to infection. The experiment was discontinued and the surviving mice sacrificed 43 days after infection. A similar experiment is shown in Table III, where an injection of cord factor (this time dissolved in olive oil) was combined with infection with various dilutions of a 3-week culture of H37Rv resulting in a chronic type of tuberculosis. It has been previously found (Bloch, 1950*b*) and confirmed (Fenner and Leach, 1953) that bacteria from 3-week old cultures are less virulent than equal numbers of organisms from young cultures of the same strain. In both sets of experiments a single injection of cord factor given one day prior to infection clearly enhanced both types of infection.

Another way of showing the enhancing effect exerted by cord factor on tuberculosis was to follow a technique used by Hart and Rees (1950) in demonstrating

TABLE II.—*Combined Effects in CF1 Mice of Cord Factor Injections and Infection with Various Doses of 5-day-old Cultures of Tubercle Bacilli.*

Group no. (10 mice per group).	Intraperitoneal injection one day before infection.	Infective dose.	Survival times of mice (days).	Median survival time (days).
1	0·1 mg. cord factor*	Undil.†	9, 9, 9, 10, 11, 13, 14, 19, 20, 20	11
2	ditto	1 : 2	9, 9, 9, 12, 15, 15, 16, 18, 19, 19	15
3	,,	1 : 4	9, 13, 14, 15, 18, 19, 21, 23, 29, 33	18
4	0·1 ml. Bayol F	Undil.	14, 14, 14, 17, 18, 19, 19, 19, 19, S‡	18
5	ditto	1 : 2	18, 18, 19, 19, 20, 20, 21, 21, 25, S	20
6	,,	1 : 4	20, 20, 20, 20, 20, 22, 22, 26, S, S	20
7	—	Undil.	16, 18, 19, 19, 19, 20, 20, 20, 20, S	19
8	—	1 : 2	18, 19, 19, 20, 20, 20, 23, S, S, S	20
9	—	1 : 4	12, 19, 21, 21, 21, 21, 21, 21, 22, S	21
10	0·1 mg. cord factor	None	2, S, S, S, S, S, S, S, S, S	> 43

* 0·1 mg. of cord factor was injected intraperitoneally, dissolved in 0·1 ml. Bayol F.

† The mice were infected intravenously with 0·1 ml. of a 5-day Tween-albumin culture of H37Rv and dilutions thereof, as indicated.

‡ S indicates mouse was sacrificed 43 days after infection, when the experiment was discontinued.

TABLE III.—*Combined Effects in CF1 Mice of Cord Factor Injections and Infection with Various Doses of 3-week-old Cultures of Tubercle Bacilli.*

Group no. (10 mice per group).	Intraperitoneal injection one day before infection.	Infective dose.	Survival times of mice (days).	Median survival time (days).
1	0·1 mg. cord factor*	Undil.†	10, 11, 15, 16, 17, 19, 20, 21, 21, 22	17
2	ditto	1 : 2	12, 13, 14, 18, 21, 22, 23, 26, 28, 29	21
3	,,	1 : 4	3, 22, 24, 27, 43, 61, S‡, S, S, S,	43
4	,,	1 : 8	7, 18, 19, 31, 42, S, S, S, S, S	42
5	,,	1 : 16	S, S, S, S, S, S, S, S, S, S	> 61
6	—	Undil.	19, 21, 23, 31, 38, 45, S, S, S, S	38
7	—	1 : 2	21, 24, 24, 29, 37, S, S, S, S, S	37
8	—	1 : 4	S, S, S, S, S, S, S, S, S, S	> 61
9	—	1 : 8	46, 52, 59, S, S, S, S, S, S, S	> 61
10	—	1 : 16	52, S, S, S, S, S, S, S, S, S	> 61
11	0·1 mg. cord factor	—	S, S, S, S, S, S, S, S, S, S	> 61

* 0·1 mg. cord factor was injected intraperitoneally, dissolved in 0·1 ml. olive oil.

† The mice were infected intravenously with 0·1 ml. of a 1/4 dilution of a 3-week-old Tween-albumin culture of H37Rv and further dilutions thereof as stated.

‡ S indicates mouse was sacrificed 61 days after infection, when the experiment was discontinued.

the enhancing effect of cortisone on chronic tuberculosis in mice. Fifty CF1 mice were injected intravenously with 0·02 ml. of a 3-week culture of H37Rv, producing chronic tuberculosis. The mice were kept together and weighed about 5 weeks after infection. The 30 animals showing the highest weights (ranging from 25 to 33 g.) were caged in equal groups of 10 and treated as follows : one group received 10 subcutaneous injections of 0·1 mg. cortisone over a period of 2 weeks ; a second group received 2 injections of 0·05 mg. cord factor with a 1-week interval between the 2 injections ; the third group was left as infection control. Two more groups of 10 non-infected mice each were added as cord factor and cortisone controls. The experiment was discontinued 120 days after infection, or 86 days after the first cord factor and cortisone injections.

The median survival time for the mice receiving cord factor was 76 days, for the cortisone-treated animals 56 days, and for the untreated controls 109 days. Thus the mice injected with cord factor died sooner than the controls, as did those injected with cortisone. The tuberculous lesions found in the lungs of the two injected groups of mice were very similar, consisting of large round necrotic areas, confluent in some parts, well isolated in others. Histologically, the necrotic areas showed amorphous débris teeming with acid-fast bacteria. While it is not suggested that the same mechanism was operative in the two groups, it appears likely that in both instances death occurred from an activation of existing tuberculous foci with a flare-up of bacterial multiplication and subsequent tissue destruction in these areas. In both groups there was a remarkably weak cellular reaction around the inflammatory centres. It did not seem unlikely that the exacerbation of chronic tuberculosis by the action of cord factor was due to a more rapid bacterial multiplication than occurred in the controls. Hence an experiment was carried out where the bacterial content of lungs, liver and spleen was determined at various times after infection in mice receiving 0·05 mg. cord factor in Bayol F prior to the infection. These values were compared to organ counts from control mice injected with 0·05 ml. of Bayol F alone. All mice were infected intravenously with a large dose of tubercle bacilli (approximately 10^8 infectious units). Three animals of each experimental group were killed by chloroform 1 hr., 2 days, 6 days and 13 days after infection. Lungs, spleens and livers were removed aseptically and the whole organs ground individually with 50 ml. of a 0·5 per cent aqueous albumin solution in a Virtis homogeniser for 30 sec. From these homogenates, subsequent dilutions and plate counts were made. The average number of bacteria cultured from the 3 corresponding organs of the animals of each group was consistently higher in the organs of the cord factor-treated mice. At 48 hr. after infection the differences were about fourfold. The initial drop in bacteria recovered within the first two days was less pronounced in the animals which had received cord factor prior to infection than it was in the control mice. Hence, the subsequent bacterial multiplication started at a higher level and rose faster to a maximum than in the controls. The differences were not of the same order in every organ. The high number of bacteria present in the liver of all mice was remarkable.

The infection-enhancing action of cord factor was effective when the compound was injected as long as 3 days before infection, but given 6 days before infection it had no noticeable effect. Just as there is a threshold dose for cord factor toxicity, the infection-enhancing effect of a single injection is dependent on the dose. Table IV lists an experiment where groups of 10 CF1 mice were given

TABLE IV.—*Effect of Various Doses of Cord Factor given 24 Hours Prior to Tuberculous Infection.**

Group no. (10 mice per group).	Amount of cord factor injected 24 hr. before infection.	Average survival time and range (days).
1	0·1 mg.	12·7 (7–18)
2	0·01 ,,	16·8 (9–24)
3	0·001 ,,	17·9 (16–20)
4	—	18·2 (17–20)

* The mice were infected intravenously with 0·1 ml. of a 5-day-old culture of H37Rv.

one injection of 0·1 mg., 0·01 mg. and 0·001 mg. respectively, 24 hr. before infection with a large dose of H37Rv. It is seen that the two lower doses had very little effect, whereas the highest dose clearly shortened the survival time. Control groups of mice receiving the cord factor injection without subsequent infection all survived throughout the observation period. They are not listed in the Table, since the failure of mice to die after one injection has been demonstrated many times before.

Specificity of the infection-enhancing action of cord factor.

The following experiments were done in order to investigate the specificity of the infection-enhancing action, both with respect to the infective agent as well as to cord factor itself. The effect of cord factor on infections with *Staph. aureus*, *Str. pneumoniae* Type III, and *Streptococcus haemolyticus* in mice were studied under exactly the same conditions as had been chosen for the analogous experiments with tubercle bacilli. Strains of relatively low virulence were used in order to have the best possible chance of observing an enhancing effect. Table V shows that cord factor had no effect on any of these infections.

TABLE V.—*Failure of Cord Factor Injections to Enhance the Effects in Mice of Pneumococcal, Staphylococcal and Streptococcal Infections.*

Number of mice per group.	Dilution of 20 hr.-culture with which mice were infected.	No. of mice dying within 5 days after infection. Group injected with 0·1 mg. cord factor prior to infection.	Control group.
15	Pneumococcus 1/10	15	14
15	,, 1/100	7	7
15	,, 1/1000	1	0
15	,, 1/10,000	1	0
10	Staphylococcus 1/10	4	8
10	,, 1/100	2	0
10	,, 1/1000	0	1
10	,, 1/10,000	0	0
10	Streptococcus 1/10	10	9
10	,, 1/100	7	8
10	,, 1/1000	1	0
10	,, 1/10,000	0	0

The specificity of cord factor was also investigated by injecting control groups, prior to tuberculous infection, with 0·1 mg. each of mycolic acid, with lipopolysaccharide (wax D), with a methylester of mycolic acid, and with a non-toxic bacterial fraction very similar to cord factor (Noll and Bloch, 1955). All compounds were derived from the same strain as the cord factor. None of these compounds, which are all chemically related to cord factor, had an enhancing effect on tuberculosis. Hence, within the limits of the experimental evidence obtained, the synergism between cord factor and tuberculosis is specific.

Interaction between cord factor and isoniazid.

It has been mentioned that isoniazid has no effect on the toxicity of cord factor, but it remained to be determined whether the drug would affect a tuber-

culous infection enhanced by cord factor. Three groups of 20 mice each were injected with 0·1 mg. of cord factor in 0·05 ml. Bayol F. On the following day, 2 groups were infected intravenously with 0·1 ml. of a 5-day culture of H37Rv ($1{\cdot}7 \times 10^7$ infectious units per dose). The third was not infected and served as control for cord factor.* Of the two infected groups, one was given a powdered pellet diet containing 0·01 per cent isoniazid for two weeks; the medication was then discontinued. The other served as control for infection combined with cord factor injection. Two additional control groups, each of 20 mice, consisted of infection controls without cord factor or isoniazid, and infection controls given isoniazid. The results are shown in Table VI. The infection-enhancing effect of cord factor was again confirmed in this experiment. More important, it is seen that this enhanced infection was little affected by isoniazid therapy, whereas the control group with no cord factor responded to the drug in the usual fashion. Thus, cord factor appears to interfere with the chemotherapeutic activity of isoniazid. This antagonistic effect between cord factor and isoniazid is dependent on the dosage of both agents. In an experiment similar to the one described in Table VI a 10 times higher dose of isoniazid (corresponding to a daily drug intake of approximately 4 mg./day/mouse) was sufficient to overcome the infection enhanced by cord factor. The antagonism between cord factor and isoniazid was observed *in vivo* only. Attempts failed to show an inhibiting effect of cord factor on the bacteriostatic action of isoniazid in cultures of tubercle bacilli.

TABLE VI.—*Interference of Cord Factor with the Therapeutic Action of Isoniazid.*

Number of mice per group.	Amount of cord factor injected 24 hr. before infection.	Dose of INH* (per cent in food).	Individual survival times (days). (Median survival time in bold type.)
20	0·1 mg.	—	7, 7, 7, 8, 8, 8, 8, 8, 8, **8**, 8, 8, 8, 9, 13, 13, 13, 13, 17, 19
20	0·1 mg.	0·01	7, 7, 7, 8, 8, 9, 11, 12, 13, **15**, 15, 15, 16, 19, 21, 31, 32, 37, 40, S†
20	—	—	17, 18, 18, 18, 18, 19, 19, 20, 20, **20**, 20, 20, 21, 21, 21, 21, 21, 23, 28, 32
20	—	0·01	19, 21, 32, S, S, S, S, S, S, S, S, S, S, S, S, S, S, S, S, S
			(Median survival time > **50**)

* INH was given as a 0·01 per cent addition to a powdered pellet diet beginning on the day of infection. The medication was discontinued after 2 weeks.

† S indicates mouse was sacrificed 50 days after infection, when the experiment was discontinued.

DISCUSSION.

Previously reported results had suggested that cord factor was concerned in the virulence of the tubercle bacillus. There was close parallelism between production of cord factor by tubercle bacilli and the pathogenicity of the organisms. Such correlation existed not only between different bacterial strains of varying virulence, but likewise between organisms of the same strain grown under varied cultural conditions affecting their virulence. These experimental findings have been recently reviewed (Bloch, 1953). The correlation which

* Not shown in Table VI. No mice of this group died during the observation period.

was consistently found under varied experimental conditions led to the conclusion that the good agreement was more than coincidental and that a definite rôle is played by cord factor in the course of a tuberculous infection. The present experiments were planned to furnish direct evidence bearing on these conclusions.

Before these experiments could be undertaken, it was necessary to have chemically pure products so that the effects observed could be safely attributed to cord factor itself and not to accompanying impurities. This goal has been achieved. Products are available, of which the chemical composition is known except for a small fragment which has not yet been fully identified (Noll and Bloch, 1955). Structurally similar products have been synthesised and were found to have qualitatively similar action (Asselineau, Bloch and Lederer, 1954). Identical cord factor preparations have been obtained in the same reproducible degree of purity from at least five different virulent strains of mammalian tubercle bacilli (Noll and Bloch, 1955). Also, chemically pure and well characterised products appeared necessary in view of the fact that biological activities of one kind or another have been attributed to so many ill-defined bacterial lipids that comparison of the results of different authors has become almost impossible.

The fact that cord factor is toxic has been a great help in assaying extracts of unknown composition, since no other related bacterial constituent with similar toxic properties was found. Although interesting in itself, the phenomenon may have limited biological significance. If cord factor enters the circulation of an animal under natural circumstances, it is always in connection with tuberculous infection and in the presence of tubercle bacilli. Therefore, the experiments where a single injection of cord factor (which as such has no lasting damaging effect) was combined with tuberculous infection have more bearing in a discussion on the rôle of cord factor. In all instances it was clear that experimental tuberculosis takes a more severe course if combined with an injection of cord factor. The injection can precede or follow an acute infection, or it can intervene later during the stationary phase of a mild infection and exacerbate the chronic disease. The degree to which the combination enhances the infection depends on the amount of cord factor injected as well as on the infective dose. As was to be expected, there is a minimal threshold dose of cord factor below which no noticeable enhancement of tuberculosis takes place, while a given effective dose of cord factor will render the mild as well as the severe experimental infections correspondingly more acute. The experimental device of combining injections of cord factor with infection is, of course, artificial, too, but there are several arguments in favour of the significance of the observed effects. First, injections of the oily medium in which cord factor was dissolved were without effect, and the activity of cord factor was the same when injected in mineral and in vegetable oil. Second, there were no indications that the mice died from toxic manifestations rather than from infection. Gross haemorrhages of lungs and liver which occur after repeated cord factor injections were absent. The mice died with the symptoms of acute tuberculosis and the gross pathological findings were in agreement with what is usually found in heavily infected animals dying after similar intervals of time. The fact that under the combination effect the mice really die of a more severe type of tuberculosis than the non-injected control animals is further brought out by the bacterial counts in various organs. Thus it seems safe to conclude that under the influence of cord factor, experimental

tuberculosis takes a more acute course than it would otherwise by rendering the local tissues more suitable to support the growth of tubercle bacilli.

The specificity of the enhancing action of cord factor on infection is indicated by the failure of related lipid constituents of the tubercle bacillus to act likewise. The so-called lipopolysaccharide or wax D which is similar to Choucroun's " Pmko " (Asselineau, Choucroun and Lederer, 1950), mycolic acid of various strains, and a non-toxic fraction structurally similar to cord factor (Noll and Bloch, 1955) did not enhance tuberculous infections. On the other hand, cord factor failed to influence infections caused by staphylococci, streptococci or pneumococci.

There are several considerations leading us to believe that cord factor is released by the tubercle bacillus *in vivo*: the compound accumulates at the bacterial surface, whence it can be extracted without killing the bacteria; only with young virulent cultures does this surface extraction yield cord factor; under appropriate conditions *in vitro*, cord factor is released into the surrounding medium. It seems reasonable to conclude, therefore, that combination effects similar to the ones observed when injecting cord factor and tubercle bacilli might naturally occur in tuberculosis at organ sites where virulent organisms multiply in tissues.

The isoniazid experiments reported in the present paper revealed an antagonistic relationship between isoniazid and cord factor which appears to be quantitative. The possibility exists that this is a specific competitive inhibition, particularly since cord factor was ineffective in similarly inhibiting the action of other chemotherapeutic agents (Bloch, unpublished work). The failure of cord factor to interfere with the bacteriostatic effect of isoniazid *in vitro* might be due to the complete insolubility of cord factor in an aqueous culture fluid. It is also possible that the antagonistic effect observed *in vivo* may be an indirect one. Cord factor produces haemorrhagic areas suitable for bacterial multiplication. Since haemin has been shown to antagonise the action of isoniazid (Fisher, 1954), the accumulation of this blood constituent in these areas might account for the relative therapeutic ineffectiveness of isoniazid in cord factor-enhanced infections.

The good correlation previously found to exist between the production of cord factor and the virulence of tubercle bacilli, the specific infection-enhancing action of cord factor shown in the present experiments, and the antagonistic effect of cord factor toward a specific antituberculosis drug, all justify the previously suggested hypothesis that cord factor plays an important part in the pathogenicity of the tubercle bacillus.

SUMMARY.

Experiments are described in which the effects of cord factor on the course of tuberculosis in mice were studied. A single injection of cord factor, which by itself has no lasting damaging effects, causes tuberculous infections to progress more rapidly than they would otherwise. Both acute and chronic infections are significantly enhanced. Mice receiving an injection of cord factor prior to infection die sooner than controls. If such mice are sacrificed at various time intervals after infection, their lungs, livers and spleens contain more viable tubercle bacilli than the organs of control animals.

The enhancing effect of cord factor on murine tuberculosis is specific. Injections of mycolic acid or bacterial wax fractions other than cord factor fail to

influence the course of experimental tuberculosis. Staphylococcal, streptococcal and pneumococcal infections in mice are not affected by injections of cord factor.

Tuberculous infections enhanced by preceding injections of cord factor respond to a much lesser degree to the therapeutic action of isoniazid. The antagonism between the drug and cord factor is observed *in vivo* only. It is quantitative in nature, depending both on the amount of cord factor injected and on the dosage of isoniazid.

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REFERENCES.

ASSELINEAU, J., BLOCH, H. AND LEDERER, E.—(1953) *Amer. Rev. Tuberc.*, **67**, 853.—(1954) *Biochim. biophys. Acta*, **15**, 136.

Idem, CHOUCROUN, N. AND LEDERER, E.—(1950) *Ibid.*, **5**, 197.

BLOCH, H.—(1950*a*) *J. exp. Med.*, **91**, 197.—(1950*b*) *Ibid.*, **92**, 507.—(1953) *Annu. Rev. Microbiol.*, **7**, 19.

Idem AND NOLL, H.—(1953*a*) *J. exp. Med.*, **97**, 1.—(1953*b*) *Trans. nat. Tuberc. Ass., N.Y.*, **49**, 94.

Idem, SORKIN, E. AND ERLENMEYER, H.—(1953) *Amer. Rev. Tuberc.*, **67**, 629.

CORNFORTH, J. W., HART, P. D'A., REES, R. J. W. AND STOCK, J. A.—(1951) *Nature, Lond.*, **168**, 150.

DUBOS, R. J. AND MIDDLEBROOK, G.—(1947) *Amer. Rev. Tuberc.*, **56**, 334.

FENNER, F. AND LEACH, R. L.—(1953) *Ibid.*, **68**, 321.

FISHER, M. W.—(1954) *Ibid.*, **69**, 469.

GOOD, R. A. AND THOMAS, L.—(1952) *J. exp. Med.*, **96**, 625.

HART, P. D'A. AND REES, R. J. W.—(1950) *Lancet*, ii, 391.

MIDDLEBROOK, G., DUBOS, R. J. AND PIERCE, C. H.—(1947) *J. exp. Med.*, **86**, 175.

NOLL, H.—(1953) *Proc. 6th Congr. int. Microbiol.*, in press.

Idem AND BLOCH, H.—(1953) *Amer. Rev. Tuberc.*, **67**, 828.—(1955) *J. biol. Chem.*, in press.

STETSON, C. A. AND GOOD, R. A.—(1951) *J. exp. Med.*, **93**, 49.