MURINE LEPROSY AND CAROTINOIDS

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While investigating *in vitro* the action of certain vegetable carotinoids on the triturated human leproma, I observed morphological modifications of the acid-resisting bacilli. This naturally led to the thought that such modifications of an infectious agent might very well represent a depreciation of its vitality, and that advantage might be obtained with the same material in experiments in the living animal. Murine leprosy was a natural field for such experiments.

The carotinoid used in this study has not yet been obtained in an absolutely pure state, for which reason I cannot as yet define its chemical structure. However, the raw primitive substance has been separated into component groups with different chemical and physical properties as regards color, physical state, solubility, and precipitation by different cations, though I have not yet been able to determine if they are chemically individual or mixtures. Of these groups of substances the following three deserve attention:

(1) Fraction A: A resinous substance, yellow, soluble in fatty solvents and also in alkaline solutions;

(2) Fraction B: Formless, intense red, with properties similar to those of fraction A;

(3) Fraction C: An oily substance, orange-red, soluble in fats and their solvents, insoluble in water or in watery alkaline solutions.

Experiments were made with these three fractions separately in the treatment of murine leprosy, in comparison with the activity of the primitive substance. A few experiments were also made with still another substance (fraction D) obtained in the laboratory through oxidation from crude carotinoid.

EXPERIMENTAL METHODS

White mice were inoculated with concentrated, triturated leproma suspension from rats infected with Stefansky's bacillus,

the injections being made under the skin in the right inguinal region. In one of the experiments the mice were injected with 0.2 cc. of infectious material, while in all of the other groups 0.3 cc. was used. In each series the animals were divided into groups, of which one served as a control and did not receive any treatment. The other groups were injected, respectively, with the raw carotinoid and fractions A, B and C. The dose of each substance was one milligram; the first three were given in 0.5 cc. of physiological serum, while fraction C was given in 0.2 cc. of olive oil. The injections were made at intervals of 5 to 7 days under the skin of the right or the left inguinal region.

Another series of mice was inoculated with 0.3 cc. of a Stefansky-bacillus suspension that had been heated at 120°C. in the autoclave for twenty minutes, so as to determine how heat-killed acid-resisting germs would behave in the organism of the mice.

The animals used in each lot of experiments were of the same age and weight and received the same food, etc. In the bacteriological examinations smears of organs stained by the Ziehl-Nielssen method were used as a standard procedure.

The results of these experiments are presented, together with statistical interpretations of the data obtained.

ANALYSIS OF RESULTS

1. CONTROL ANIMALS INOCULATED WITH LIVING AND HEAT-KILLED BACILLI

First to be considered is the distribution of the microorganism in the organs of the control animals, which were not given any injections after inoculation. In Table 1 are shown the findings in the group inoculated with living germs, these animals having been killed and examined at intervals varying from 30 to 80 days after inoculation. It will be seen that under the conditions of the experiment there was not a universal distribution of the germs in the organs examined.

At a glance Table 2, in which are given the findings after from 8 to 80 days in the animals inoculated with the heat-killed suspension, supplies us with much more important information. It shows that even the dead germs may be found in the greater part of the organs examined.

With these early observations in hand it became necessary to ascertain the frequency of the presence of bacilli in the var-

ious organs of the animals inoculated with live germs and those killed by heat. In the event of an equal frequency of positivity in both cases it would have to be decided whether the presence of germs in the various organs could be attributed to the peculiar activity of those organs, or would have to be explained in a different manner; in any case the bacteriological diagnosis could not give an indication as to whether a therapeutic measure, directed against the bacillary activity, was efficient or not. Conversely, for a bacteriological diagnosis, a proportional importance between the difference of the probability of positivity through inoculation of live germs and the probability of positivity through inoculation of germs killed by heat is essential.

In order that the determination of frequency may be rigorous, the results of experiments should be in accordance with predetermined conditions, such as the maintaining of constant intervals between the date of inoculation of the germs, whether living or dead, and the examination of the animals. Only in this manner can the presence of germs in the various organs be proved to be independent of time.

If these preliminary conditions are not observed, in a number of cases sufficient for statistical analysis, we must admit the hypothesis of equality of all results, independent of the time at which they were obtained. That, it must be admitted, can only lead to approximate results, and any conclusion cannot be more than a simple indication. This restriction is still further justified when the limited number of observations is considered, but such a procedure justifies itself when it is taken into consideration that this report is of preliminary character, to orientate future studies.

By adopting this orientation there were obtained, for the 13 rodents inoculated with live germs and in 6 inoculated with killed ones, the percentages of positivity in the various organs given in Table 3. Analyzing the results there shown we see that the difference of frequency of positive findings in the liver in the two cases cannot be attributed to the mere fluctuations of simple chance, as the difference is three times as great as its standard error. This would place the liver as the most important organ in the evaluation of a bacteriological diagnosis. It is true that, with certain reservations, the spleen could also fulfil this function, in view of the fact that the quotient between the percentage difference and its standard error is 2.24.

Once it is established within the adopted criteria that the percentage of positive results in the livers of the mice inoculated with live germs is greater than that of those inoculated with germs killed by heat, we may go on to ascertain whether or not identical results may be obtained from animals that were inoculated with live germs and which did not receive treatment, when compared with those that were similarly inoculated but which received treatments of different kinds. Although in this connection the findings obtained in the various regions are given, special attention is invited to the results of the examinations of the liver and spleen.

2. ANIMALS TREATED WITH CRUDE CAROTINOID

The frequency with which bacilli were found in the different tissues of the inoculated animals (each of which received 0.3 cc. of the leproma suspension) that were treated with the crude carotinoid is shown in Table 4. Adopting the same criteria as for the previous examination—in other words considering the results alike independently of the number of days between inoculation and the autopsy and also independently of the dose of the drug given—we may compare the positivity frequency of this table with that of the untreated injected animals shown in Table 1. It will be noted that the periods of both tables are similar. This comparison is shown in Table 5. Here again will be seen a greater percentage of positivity of the liver and spleen in the untreated animals as compared with the treated ones, a difference which according to the criteria adopted cannot be explained by fluctuations of simple samples. It will also be seen that among the untreated rodents there was a materially greater positivity percentage for the left axillary lymph nodes, the difference being 2.17 times as great as the standard error.

To ascertain a possible equivalent between the frequencies of the results of those treated with crude carotin and those inoculated with heat-killed germs, Table 6 has been prepared in a similar way. As can be seen, every difference between these two groups may be explained on the ground of fluctuations of the simple samples. For the two instances in which there is the most possibility of there being an actual significance, the degree of positivity is greater for those rodents inoculated with sterilized germs in the case of the right axillary lymph node, and smaller for those in the case of the spleen.

3. ANIMALS TREATED WITH FRACTION A.

The findings in the infected animals treated with fraction A are given in Table 7. As with the preceding group, these findings are compared statistically with those in the untreated infected controls, in Table 8, and with the animals inoculated with killed bacilli, in Table 9.

These data show, again, a greater degree of positivity in the untreated infected mice as compared with those treated with substance A. The difference observed with respect to the spleen can be accepted as independent of the fluctuations of chance, and the difference with respect to the liver is very possibly significant. On the other hand the differences between this treated group and the one inoculated with heat killed germs may again be explained by fluctuations of samples. This result therefore is very similar to that obtained with the raw carotinoid.

4. ANIMALS TREATED WITH FRACTION B.

The findings in the group of rodents treated with substance B are given in Table 10, and they are analyzed as before in Tables 11 and 12. Table 11 shows a great similarity in percentages of positivity in the treated and the untreated infected groups. It will be noted that the liver and the spleen were positive most frequently in the untreated animals, but any conclusion from those figures would be unwarranted. Table 12 indicates that the difference between the percentages of positivity in the liver of those inoculated with sterilized germs and those that were infected and treated may very possibly be nearly correct.

5. ANIMALS TREATED WITH FRACTION C.

The findings in this group of animals are dealt with as in the preceding sections, in Tables 13, 14 and 15. It will be noted that the percentages of positivity of liver and spleen are greater in the animals which were not treated than in those which were treated with substance C, the differences observed having some degree of accuracy, but at the same time the percentages of positivity in those organs of the rodents so treated are nevertheless greater than corresponding percentages in those inoculated with sterilized germs, the differences also having some degree of accuracy.

6. ANIMALS TREATED WITH FRACTION D.

The results obtained in three mice treated with substance

D are given in Table 16. They will not be considered in detail because of the small number of observations.

DISCUSSION

Summing up the results of the complete analysis, attention is drawn to the following points:

(1) With the exception of the right inguinal lymph node (i.e., the one on the side of injection), inoculation with live germs was not systematically followed by positive findings in all of the regions examined in the bacteriological examinations, which were made at intervals that varied between 30 and 80 days after the inoculation.

(2) With the exception of the liver, inoculation with germs killed by heating at 120°C. is not systematically followed by negative bacteriological findings in all of the regions examined.

(3) It may be affirmed that, within the limits of the criteria adopted, the frequency of positive bacteriological findings is significantly higher in the livers of those mice inoculated with live germs than in those inoculated with dead ones. The same is probably true of the spleens.

(4) The frequency of positive findings in the liver and spleen is greater in rodents inoculated with live germs and not treated than in the group that was injected with crude carotinoid.

(5) Almost exactly the same results were obtained in the group treated with substance A as in that in which the crude carotinoid was used.

(6) It would be going too far, on the other hand, to assert that similar findings were obtained in the groups treated with substances B and C.

It was observed that, in the series of mice treated, the number found negative in the examinations of the spleens and livers was not small, although lymph nodes examined were positive. It would be well to connect this fact with what was observed in rodents inoculated with sterilized bacilli. It is evident that, when dead acid-resisting bacilli are injected under the skin of a mouse, they enter the lymphatic circulation and reach a greater or a lesser number of lymph nodes. At the same time they suffer phagocytosis, and by means of the phagocytes they reach the blood stream, thus being distributed to the various viscera. The spleen, on the whole, is more liable than the liver to receive such phagocytized germs, because of

the lymphoid and lacunar structure of the pulp and also its type of physiological activity (lymphopoietic organ). In the case of live germs, the probability of their being found in the spleen and liver is still greater, in the first place because they multiply and increase in numbers, and in the second place because those of filterable quality would probably not be as subject as the others to the retention mechanism of the lymph nodes. The finding of acid-resisting germs in the nose—an examination which was made to determine so far as possible the state of the nasal mucosa—has no real value, for it was learned later than general saprophytic germs with acid-resisting characteristics may be found in the nasal mucus of rats in consequence of their eating cereal foods.

It is clear that organic detritus in a live organism will generally undergo a process of decomposition, which will occur either as a result of activity of the organism itself (an enzymic process) or by means of actual autolysis. A bacterial cell made up of protein substances should undergo destruction by this double method and be eliminated. This lysis is generally a rapid process. In the case of acid-resisting germs, however, perhaps because of the nature of their membrane, the host organism is incapable of destroying them, and they also fail to undergo autolysis. Thus in the elimination of the dead acid-resisting germs the organism will not behave in the usual manner, as with other germs and with organic substances in general; the method is closer to that of eliminating inorganic particles, and perhaps to a certain extent it comes between the two with respect to the time needed for the elimination.

It is, therefore, obvious that the presence of acid-resisting germs in the lymph nodes and in visceral organs of rodents inoculated with the microorganism does not indicate that the germs are actually invading or that those which are found there have vitality. An efficient treatment against a disease caused by such germs cannot make the bacterial elements disappear from the organism, though it definitely interrupts the evolution of the disease. It is true that this condition is of no value with regard to open lesions; in that event a process of mechanical elimination is required, as in the cicatrization of the tuberculous lesions of a lung or of the involution processes of leprosy, either of the skin or the nasal mucosa.

On the other hand a comparison between the examinations of the liver and spleen of the rodents inoculated with heat-

killed Stefansky bacilli and of those inoculated with live microbes proves satisfactorily that, in the latter case, there was a real invasion by the germs, which localized in the viscera mentioned to produce a pathological state, while the dead microorganisms are transported to the spleen passively, with the probable intervention of the cells of the reticulo-endothelium. If, therefore, one wishes to judge the efficiency of a treatment of murine leprosy one will have to adopt as criteria: (*a*) when it is desired to demonstrate an inhibitory effect on the microorganism, examination of the liver and spleen is required, not of the peripheral lymph nodes; and (*b*) to judge the curative effect, one must depend upon either the anatomo-pathological examination or on subinoculation of healthy rodents, and not on the bacteriological examination.

There is no doubt, therefore, that to interpret our results we should place value only upon the figures obtained from the examinations of the liver and spleen. On that basis we see that the animals which were treated with raw carotinoid and with substance A gave exactly the same results as if they had been inoculated with dead germs. Logically, therefore, a positive effect in the treatment of murine leprosy can be attributed to these two substances.

As for substances B and C, the small activity which they showed perhaps rests not only in a possible contamination with the active substance A, but also—this in the case of fraction C —because it was used in the form of an oily solution of slight absorbability in the rats. It seems to me probable that fraction C represents a forerunner of fraction A, and I believe that the change of the one into the other is made by a partial oxidation phenomena and is not reversible. The fourth fraction (D) which was tested in a few animals and found to be active may be the actual substance C artificially changed into component A. Attention should be called to the fact that the efficient quantities of the active substances were always extraordinarily small—3 to 5 milligrams of A, and 6 to 15 of the raw carotinoid. These amounts were used in experiments in which the minimum capacity of activity was not sought. It is not impossible that the minimum may be found in still more limited quantities.

SUMMARY

In this study of the effect of substances of the carotinoid type in the treatment of murine leprosy, it has been observed

that there is a difference in the distribution of the Stefansky bacillus in the organism of mice according to whether the germs used in the inoculation are alive or have been killed by heat (autoclaving at 120°C. for 20 minutes). Within the period of 80 days after inoculation, pieces of the liver were always negative when the inoculated germ was dead, and positive in the majority of cases when the germ was alive. Similar though less clear-cut findings were obtained with regard to the spleen. With infected animals treated with a crude substance of carotinoid type it was observed that the distribution of bacilli was the same as if they had been inoculated with dead germs, and that was also true when the treatment was with one of the fragments (fraction A) of the primitive substance. Two other fractions (B and C) were inactive, although it would appear probable that the active substance (fraction A) originates through an oxidation process from fraction C. A substance obtained in the laboratory through oxidation of raw carotinoid (fraction D) showed activity in a small number of observations.

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TABLE 1. *Distribution of bacilli in mice inoculated with living Stefansky bacilli.*

Region examined	Findings in animals examined (days after inoculation)												
	30[a]			30	48	55	63	70	75		80		
Right inguinal node	+	+	+	+	+	+	+	+	+	+	+	+	+
Left inguinal node	+	+	+	+	+	+	+	+	+	—	+	+	+
Right axillary node	+	+	+	+	+	+	+	+	+	+	—	—	+
Left axillary node	—	+	+	—	+	+	+	+	—	+	—	+	+
Nose (incision)	+	—	—	—	+	—	—	—	—	+	+	+	—
Liver	+	+	+	+	+	—	+	—	+	+	—	+	+
Spleen	+	+	—	+	+	+	+	+	+	+	—	+	+

[a] The animals represented here received 0.2 cc. of the suspension, the others 0.3 cc.

TABLE 2.—*Distribution of bacilli in mice inoculated with a heat-killed suspension of Stefansky bacilli.*

Region examined	Findings in animals examined (days after inoculation)					
	8	18	28	40	65	80
Right inguinal node.....	+	+	+	+	+	+
Left inguinal node	+	+	+	+	+	—
Right axillary node	+	—	+	+	—	+
Left axillary node	+	—	—	+	—	+
Nose (incision)	+	—	—	—	+	+
Liver	—	—	—	—	—	—
Spleen	—	—	—	+	+	—

TABLE 3.—*Statistical analysis of the findings in mice inoculated with living and with heat-killed bacilli.*

Region examined	Frequency of positive findings		Difference between (1) and (2) (3)	Standard error of difference (4)[a]	(3)/(4)
	Live bacilli inoculated (1)	Killed bacilli inoculated (2)			
Right inguinal node..	100.00%	100.00%	0	—	—
Left inguinal node...	92.31%	83.33%	8.98%	15.14%	0.59
Right axillary node..	84.62%	66.67%	17.95%	20.19%	0.89
Left axillary node....	69.93%	50.00%	19.23%	23.81%	0.81
Nose (incision).....	38.69%	50.00%	11.38%	24.37%	0.47
Liver.....	76.92%	0.00%	76.92%	24.64%	3.12
Spleen.....	84.62%	33.33%	51.29%	22.94%	2.24

[a] The results given in column 4, and in corresponding columns of other tables to follow, were obtained by supposing the condition of simplicity of samples to be fulfilled.

TABLE 4. *Distribution of bacilli in infected mice treated with crude carotinoid.*

Region examined	Findings in animals examined (days after inoculation and, in parentheses, amounts of the drug given, in milligrams)								
	30 (9)	48 (6)	55 (6)	63 (8)	70 (9)	75 (9)	75 (15)	80 (9)	80 (15)
Right inguinal node	+	+	+	+	+	+	+	+	+
Left inguinal node	—	+	+	+	+	+	—	+	+
Right axillary node	+	+	+	+	+	+	+	+	+
Left axillary node.....	—	—	+	—	—	—	—	+	—
Nose (incision).....	—	+	—	—	—	+	—	—	+
Liver.....	—	—	—	—	+	—	—	—	—
Spleen.....	—	—	—	—	—	—	—	—	—

TABLE 5.—*Statistical analysis of the positive findings in* (a) *the infected mice treated with raw carotinoid and* (b) *the untreated infected controls.*

Region examined	Frequency of positive findings		Difference between (1) and (2) (3)	Standard error of difference (4)	(3)/(4)
	Untreated controls (1)	Treated animals (2)			
Right inguinal node..	100.00%	100.00%	0	—	—
Left inguinal node...	92.31%	77.78%	14.53%	14.75%	0.99
Right axillary node..	84.62%	100.00%	15.38%	12.47%	1.23
Left axillary node....	69.23%	22.22%	47.01%	21.68%	2.17
Nose (incision).....	38.62%	33.33%	5.29%	20.86%	0.25
Liver.....	76.92%	11.11%	65.81%	21.68%	3.04
Spleen.....	84.62%	0.00%	84.62%	21.68%	3.90

TABLE 6.—*Statistical analysis of the positive findings in* (a) *the infected mice treated with raw carotinoid and* (b) *the controls inoculated with heat-killed bacilli.*

Region examined	Frequency of positive findings		Difference between (1) and (2) (3)	Standard error of difference (4)	(3)/(4)
	Control animals (1)	Treated animals (2)			
Right inguinal node..	100.00%	100.00%	0.00%	—	—
Left inguinal node...	83.33%	77.78%	5.55%	21.08%	0.26
Right axillary node..	66.67%	100.00%	33.33%	17.91%	1.86
Left axillary node....	50.00%	22.22%	27.78%	24.84%	1.12
Nose (incision).....	50.00%	33.33%	16.67%	25.81%	0.65
Liver.....	0.00%	11.11%	11.11%	13.14%	0.85
Spleen.....	33.33%	0.00%	33.33%	17.91%	1.86

TABLE 7.—*Distribution of bacilli in infected mice treated with fraction A.*

Region examined	Findings in animals examined (days after inoculation and, in parentheses, amounts of the drug given, in milligrams)													
	30[a]			30	48	55	63	70	75		80			
	(3)	(3)	(3)	(9)	(6)	(6)	(8)	(9)	(9)	(15)	(9)	(15)	(15)	(15)
Right inguinal node	+	+	+	+	+	+	+	+	+	+	+	+	+	—
Left inguinal node	+	+	+	—	—	+	+	+	—	+	+	—	—	+
Left axillary node	—	+	—	+	+	+	+	+	+	—	+	+	+	—
Left axillary node	—	+	+	—	—	+	—	+	—	—	+	—	—	+
Nose (incision)	+	—	—	—	—	+	—	+	—	—	—	+	—	+
Liver	—	—	—	—	+	—	—	+	—	—	—	—	—	+
Spleen	—	—	—	—	—	+	—	+	—	—	—	—	—	—

[a] The animals represented here received 0.2 cc. of the suspension, the others 0.3 cc.

TABLE 8.—*Statistical analysis of the positive findings in* (a) *the infected mice treated with fraction A and* (b) *the untreated infected controls.*

Region examined	Frequency of positive findings		Difference between (1) and (2) (3)	Standard error of difference (4)	(3)/(4)
	Untreated controls (1)	Treated animals (2)			
Right inguinal node..	100.00%	92.86%	7.14%	7.27%	0.98
Left inguinal node...	92.31%	64.29%	28.02%	16.01%	1.75
Right axillary node..	84.62%	71.43%	13.19%	16.01%	0.82
Left axillary node....	69.23%	42.86%	26.37%	19.13%	1.38
Nose (incision).....	38.62%	28.57%	10.05%	18.15%	0.55
Liver.....	76.92%	21.43%	55.49%	19.24%	2.88
Spleen.....	84.62%	14.29%	70.33%	19.24%	3.66

TABLE 9.—*Statistical analysis of the positive findings in* (a) *the infected mice treated with fraction A and* (b) *the controls inoculated with heat-killed bacilli.*

Region examined	Frequency of positive findings		Difference between (1) and (2) (3)	Standard error of difference (4)	(3)/(4)
	Untreated controls (1)	Treated animals (2)			
Right inguinal node..	100.00%	92.96%	7.14%	10.63%	0.67
Left inguinal node...	83.33%	64.29%	19.04%	22.36%	0.85
Right axillary node..	66.67%	71.43%	4.76%	22.36%	0.21
Left axillary node....	50.00%	42.86%	7.14%	24.28%	0.29
Nose (incision).....	50.00%	28.57%	21.43%	23.27%	0.92
Liver.....	0.00%	21.43%	21.43%	17.42%	1.23
Spleen.....	33.33%	14.29%	19.04%	19.52%	0.98

TABLE 10.—*Distribution of bacilli in infected mice treated with fraction B.*

Region examined	Findings in animals examined (days after inoculation and, in parentheses, amounts of the drug given, in milligrams)													
	30[a]		30	48	55	63	70	75		80				
	(3)	(3)	(3)	(9)	(6)	(6)	(8)	(9)	(9)	(15)	(9)	(15)	(15)	(15)
Right inguinal node	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Left inguinal node	+	+	+	+	+	+	+	+	+	+	+	+	—	+
Right axillary node	+	+	+	+	+	+	+	+	+	—	+	—	+	+
Left axillary node	+	+	—	—	—	+	—	—	+	+	+	—	+	—
Nose (incision)	+	+	+	—	—	—	—	—	—	+	+	—	+	—
Liver	—	+	+	—	+	—	—	+	—	—	+	+	+	+
Spleen	—	+	+	+	+	+	+	+	+	—	+	+	—	—

[a] The animals represented here received 0.2 cc. of the suspension, the others 0.3 cc.

TABLE 11.—*Statistical analysis of the positive findings in* (a) *infected mice treated with fraction B and* (b) *the untreated infected controls.*

Region examined	Frequency of positive findings		Difference between (1) and (2) (3)	Standard error of difference (4)	(3)/(4)
	Untreated controls (1)	Treated animals (2)			
Right inguinal node..	100.00%	100.00%	0	—	—
Left inguinal node....	92.31%	92.86%	0.55%	10.09%	0.05
Right axillary node..	84.62%	85.71%	1.09%	13.68%	0.08
Left axillary node....	69.23%	50.00%	19.23%	18.93%	1.02
Nose (incision).....	38.62%	22.86%	4.24%	18.93%	0.22
Liver.....	76.92%	57.14%	19.78%	18.16%	1.09
Spleen.....	84.62%	71.43%	13.19%	16.01%	0.82

TABLE 12.—*Statistical analysis of the positive findings in* (a) *the infected mice treated with fraction B and* (b) *the controls inoculated with heat-killed bacilli.*

Region examined	Frequency of positive findings		Difference between (1) and (2) (3)	Standard of error difference (4)	(3)/(4)
	Control animals (1)	Treated animals (2)			
Right inguinal node...	100.00%	100.00%	0	—	—
Left inguinal node...	83.33%	92.86%	9.53%	14.64%	0.65
Right axillary node...	66.67%	85.71%	19.04%	19.52%	0.98
Left axillary node...	50.00%	50.00%	0	—	—
Nose (incision).....	50.00%	42.86%	7.14%	24.28%	0.29
Liver.....	0.00%	57.14%	57.14%	23.90%	2.39
Spleen.....	53.33%	71.43%	38.10%	24.28%	1.57

TABLE 13.—*Distribution of bacilli in infected mice treated with fraction C.*

Region examined	Findings in animals examined (days after inoculation and, in parentheses, amounts of the drug given, in milligrams)							
	30[a] (3)		48 (6)	55 (6)	63 (8)	70 (9)	75 (9)	80 (9)
Right inguinal node.....	+	+	+	+	+	+	+	+
Left inguinal node.....	+	+	+	—	+	—	+	+
Right axillary node.....	+	+	+	+	+	—	+	+
Left axillary node.....	+	+	+	—	+	—	—	+
Nose (incision).....	+	+	+	—	+	—	+	—
Liver	+	+	—	—	+	—	—	—
Spleen	+	+	+	—	+	—	+	—

[a] The animals represented here received 0.2 cc. of the suspension, the others 0.3 cc.

TABLE 14.—*Statistical analysis of the positive findings in* (a) *the infected mice treated with fraction C and* (b) *the untreated infected controls.*

Region examined	Frequency of positive findings		Difference between (1) and (2) (3)	Standard of error difference (4)	(3)/(4)
	Untreated controls (1)	Treated animals (2)			
Right inguinal node...	100.00%	100.00%	0	—	—
Left inguinal node....	92.31%	75.00%	17.31%	15.73%	1.10
Right axillary node...	84.62%	87.50%	2.88%	15.73%	0.18
Left axillary node	69.23%	62.50%	6.73%	21.18%	0.32
Nose (incision)	38.62%	25.00%	13.62%	21.18%	0.64
Liver	76.92%	37.50%	39.42%	21.82%	1.81
Spleen	84.62%	62.50%	22.12%	19.14%	1.16

TABLE 15.—*Statistical analysis of the positive findings in* (a) *the infected mice treated with fraction C and* (b) *the controls inoculated with heat-killed bacilli.*

Region examined	Frequency of positive findings		Difference between (1) and (2) (3)	Standard error of difference (4)	(3)/(4)
	Control animals (1)	Treated animals (2)			
Right inguinal node..	100.00%	100.00%	0	—	—
Left inguinal node...	93.33%	75.00%	8.33%	22.16%	0.38
Right axillary node..	66.67%	87.50%	20.83%	22.16%	0.94
Left axillary node....	50.00%	62.50%	12.50%	26.73%	0.47
Nose (incision).....	50.00%	25.00%	25.00%	25.88%	0.97
Liver.....	0.00%	37.50%	37.50%	22.16%	1.69
Spleen.....	33.33%	62.50%	29.17%	27.00%	1.08

TABLE 16.—*Distribution of bacilli in infected mice treated with fraction D.*

Region examined	Findings in animals examined (days after inoculation and, in parentheses, amounts of the drug given, in milligrams)		
	48 (2)	63 (5)	75 (7)
Right inguinal node.....	+	+	+
Left inguinal node.....	—	+	+
Right axillary node.....	+	+	+
Left axillary node.....	—	+	+
Nose (incision).....	—	—	—
Liver.....	—	—	—
Spleen.....	—	—	+