

THE DEGREE OF DISPERSION OF THE BACILLUS AS A
FACTOR IN INFECTION AND RESISTANCE IN
EXPERIMENTAL TUBERCULOSIS

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PLATES 1 TO 3

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Aqueous extracts of testicle contain a substance which produces a rapid and pronounced increase in the permeability of the dermis and connective tissues for injected fluids and suspensions (1-3). Toxins, suspensions of bacteria, and viruses may, with the aid of such a spreading agent, be dispersed through a greatly increased area of skin, and this dispersion brings about definite alterations in the nature of the resulting lesions (1, 4). In the case of toxins, and of antigens injected into previously sensitized animals, the dispersion of the material brings about a diminution of the intensity of the reaction, along with an increase in the area or extent of the lesion. The dispersion of large amounts of living bacteria augments both the size and severity of the lesion which develops, as compared with that produced by like numbers of bacteria injected in saline solution.

It was found (5) that when progressively smaller amounts of bacteria were injected together with extracts of testicle or of invasive staphylococci, the lesions were enhanced as a result of spreading, when their number in the suspension was above a minimal effective concentration; and were partially or totally suppressed when their number in the suspension fell below such a concentration. The virulence of the bacteria and the minimal effective concentration were found to be inversely related. Contrarily, the lesions produced by filterable viruses, even in quantities closely approaching the minimal infective dose, were enhanced by the dispersing factor.

The present paper deals with the effects of such dispersion on tuberculous infection in guinea pigs and rabbits. As in the case of other

bacteria, the extent of dispersion of injected tubercle bacilli is an important factor in the development of the primary infection as well as in the partial resistance elicited by reinfection, and in the resistance developed by vaccination with killed organisms.

The spreading agent used throughout this investigation was testicle extract. The fact that this extract acts by markedly increasing the permeability of the tissues has been proven in a series of studies (5-8). We have no evidence whatsoever that it has any direct action on any of the infectious agents themselves.

Walker and Hoffman (9) have already reported that intradermal injection into rabbits of testicle extract together with suspensions of human, bovine, and avian strains of tubercle bacilli resulted in more widespread lesions. Some of our own studies on the effect of testicle extract on tuberculous infection of guinea pigs have already been briefly reported (10).

Material and Methods

Characteristics of the Strains of Mycobacteria Employed

M. tuberculosis (Human Type, Strain H 37).—This strain is of moderate virulence for rabbits when injected intravenously, producing a slowly progressive disease. Rabbits are markedly resistant to it when injection is made into or beneath the skin, practically never developing generalized lesions. Guinea pigs on the other hand are quite susceptible to infection with it, developing far advanced tuberculosis within 90 days after subcutaneous inoculation with 0.1 mg. or less.

M. tuberculosis (Bovine Type, Strain B-1).—This strain of bovine tubercle bacilli is but moderately virulent for rabbits, producing a very slowly progressive disease after intravenous inoculation.

M. tuberculosis (Bovine Type, Strain Ravenel).—This strain is highly virulent for rabbits, producing a rapidly progressive widespread disease after intravenous inoculation.

M. tuberculosis (Avian Type, Strain Avian 1).—This strain is avirulent for both rabbits and chickens at the present time, although 3 years ago it was highly virulent for both.

B.C.G.—This is the well known bacillus of Calmette and Guérin (used for prophylactic vaccination). It is avirulent for all laboratory animals. It was kindly supplied by the New York City Board of Health.

Defatted Bovine Tubercle Bacilli.—These organisms had been extracted with alcohol and ether for 2 months, following which they were dried and ground.

Preparation of Suspensions of Tubercle Bacilli

Cultures grown on Corper's egg medium for 3 to 6 weeks were used. A suitable amount of organisms were harvested from the surface of the medium and weighed immediately. They were then triturated in a mortar with saline solution and finally brought to a concentration of 1.0 mg. of bacteria per cc. The addition of different volumes of saline solution to aliquot portions of the concentrated bacterial suspension provided a graded series of dilutions of bacteria. When heat-killed bacteria were employed they were prepared by immersing the flask containing the suspension in a water bath at 70°C. for 1 hour.

Preparation of the Spreading Agent

Testicle extract was prepared from bull testicles. The membranes were stripped from the fresh organs, which were then minced and ground with sand and 4 volumes of 0.9 per cent saline solution. After centrifugation the extract was filtered through a Berkefeld V candle and then through a Berkefeld W candle. The extract was put into test tubes and stored in the refrigerator.

Method of Testing the Effect of Spreading of Tubercle Bacilli

Mixtures were prepared generally containing 0.5 or 1.0 cc. of a bacterial suspension and testicle extract. These were injected into the skin of rabbits and guinea pigs. Similar suspensions containing saline solution instead of testicle extract were always injected as a control either into the same or another animal. When trying the effect of spreading different dilutions of bacillary suspensions in rabbits, no more than 5 injections were made on one side.

The injection of mixtures containing testicle extract was followed in both rabbits and guinea pigs by a marked spreading, as evidenced by the prompt flattening and disappearance of the wheal, and by the extent of the resulting edema and blanching.

Mature rabbits bred at The Rockefeller Institute from Lilac and English stock were used. Guinea pigs weighing from 250 to 500 gm. were obtained from outside sources.

Recording of the Results

Measurements and colored drawings were made of skin lesions at frequent intervals. The lesions were described in terms of the area of skin affected and also the intensity of the inflammation. The extent of tuberculous involvement found at autopsy was recorded according to the following plan. The inguinal, axillary, cervical, tracheal, mesenteric, and iliac lymph nodes were removed cleanly and weighed, and estimates of their involvement made. The spleen was weighed. The extent of the gross pulmonary, hepatic, and renal tuberculosis was estimated and graded from 1 to 4, grade 1 representing minimal involvement and grade 4 maximum involvement. Histological sections were made as a routine procedure.

The Effect of Testicle Extract on Experimental Tuberculosis in Rabbits

The effect of dispersion on the lesions induced by intradermal infection was studied with several strains of tubercle bacilli available, which differed markedly in their virulence for rabbits.

Infection with Bovine Tubercle Bacilli, Strain B-1.—3 rabbits were injected in the skin of the flank with 1.0 mg. of B-1 cultures suspended in 2.0 cc. of testicle extract, and 3 additional rabbits were similarly injected using saline solution instead of testicle extract.

4 to 6 days after the inoculation the animals injected with the saline suspension developed small nodules at the site of inoculation, and by 16 days these had increased slightly in size and ulceration and drainage of purulent material occurred. These lesions persisted during the 7 weeks of observation, although becoming slightly smaller and less productive after the 4th week.

The 3 animals injected with the testicle extract suspension showed a marked inflammatory reaction in the skin 4 days after inoculation, about 10 times as large as in the controls. During the next 8 to 10 days the inflammatory reaction increased in severity, the skin became indurated, and the central portions underwent necrosis. Following this the entire lesion became ulcerated. Seropurulent material drained from these ulcers for approximately 10 days, after which they became covered with apparently healthy granulation tissue. During the next 4 weeks of observation these ulcers diminished considerably in size. The skin forming the border of these ulcers was not normal healthy skin, however, as in many places small pockets of caseous material had formed. Such lesions are illustrated in Figs. 1, 2, and 3.

The condition of these animals during the 7 weeks of observation was in marked contrast to the 3 control animals injected with the saline suspension. They lost an average of 540 gm. in weight, whereas 2 of the controls maintained their original weight and one registered a slight gain.

50 days after infection the 6 animals were sacrificed. The 3 control animals showed involvement of the regional lymph glands, but no visceral lesions. On the other hand, all of the animals injected with the testicle extract suspension had developed pulmonary tuberculosis, in one instance associated with splenic tuberculosis, and in another with tuberculosis of the testicles.

Infection with Bovine Type Bacilli, Strain Ravenel.—2 rabbits were injected in the skin of the flank with 1.0 mg. of Ravenel culture suspended in 2.0 cc. of testicle extract. 2 additional control rabbits were similarly injected with 1.0 mg. of bacilli suspended in 2.0 cc. of physiological salt solution.

The resulting lesions were similar in nature and in their rate of development to the lesions which followed inoculation with the B-1 strain. The animals lost weight during the 50 days of observation, although the 2 injected with testicle extract lost considerably more than the 2 controls. At the end of this time they were sacrificed. The 2 control animals showed a few scattered tubercles in the lungs and kidneys. The 2 animals that had been injected with testicle extract showed widespread pulmonary tuberculosis, extensive renal tuberculosis, and tubercles in the spleen and bone marrow.

Infection with the Human Strain H 37.—6 rabbits were injected in the skin of the flank with 1.0 mg. of H 37 bacilli suspended in 2.0 cc. of testicle extract. 3 control rabbits were similarly injected with 1.0 mg. of H 37 bacilli suspended in 2.0 cc. of saline solution.

The skin lesions which developed in these animals were similar to those following inoculation with the 2 bovine strains. However, they developed more quickly and also reached their maximum size earlier. After 7 weeks of observation the entire group was sacrificed. It was found that in none of the animals was there any evidence of visceral infection, although extension of the infection to the local or regional lymph glands had occurred in all.

Infection with Avian Type Bacilli, Strain Avian 1.—3 rabbits were injected in the skin of the flank with 2.0 mg. of Avian 1 bacilli suspended in testicle extract, and 3 control rabbits were injected with a similar dose suspended in saline solution.

The lesions resulting from the injection of the testicle extract suspension were markedly increased in size and severity as in the preceding experiments with the human and bovine strains. The increased dose (2.0 mg. instead of 1.0 mg. as in the three preceding experiments) resulted in a more severe local lesion, with ulceration extending into the subcutaneous tissues. The lesions developed more rapidly than those following inoculation with either the human or the bovine

strain. However, when the 6 animals were sacrificed at the end of 4 weeks, no visceral tuberculosis was found in any of them.

Infection with B.C.G.—2 rabbits were injected in the skin of the flank with a suspension of 1.0 mg. of B.C.G. in 2.0 cc. of testicle extract, and 2 control rabbits were similarly injected with a suspension in saline solution.

In the animals injected with the suspension in testicle extract there was a marked enhancement of the local lesion, and involvement of the regional lymph nodes, but in no case was there any evidence of extension to the lungs or other viscera.

The foregoing experiments showed that the intradermal injection of relatively large amounts of tubercle bacilli suspended in testicle extract resulted in a marked enhancement of the local lesion. This finding is in agreement with observations made on other bacteria and viruses.

Enhancement of the local lesion was followed by an increase in the visceral lesions, in the case of the two virulent bovine strains. The other strains employed were not capable of producing generalized lesions when the amount used was injected into the skin of rabbits without testicle extract. The addition of this agent did not enable such visceral infection to take place.

The Dermal Lesions Produced in Rabbits by the Simultaneous Injection of Testicle Extract and Small Quantities of Tubercle Bacilli

Experiments with Living Bacilli.—It has been shown with many bacterial strains that when the number of bacteria injected falls below a certain critical concentration, the action of testicle extract results in the suppression of the lesion rather than an enhancement of it. Experiments were devised to study the possibility of such suppression of lesions in the case of tubercle bacilli.

Suspensions of 5 acid-fast strains were prepared as usual. Each strain was tested by intradermal injection in several dilutions with testicle extract on one flank of a rabbit, and on the other side the same dilutions made with normal saline solution were tested. Each test was repeated on 2 or more animals. The strains used, the quantity of bacteria injected, and the average size and intensity of the lesions resulting in a total of 15 rabbits are given in Table I.

It will be seen from Table I that the spreading action of testicle extract enhances the lesions in the skin in practically all of the dilutions tested, even in those which approach the minimal infective dose, and that this occurred in all of the strains studied. The severity of the lesions produced in the skin by the various strains bears no relation to their ability to invade the tissues of the host.

In two additional tests dealing with the H 37 strain of human tubercle bacilli, and with the Avian 1 strain, each of the dilutions was tested in a single rabbit, thus eliminating the possibility that the production of several lesions in one rabbit would result in interference or anomalous results. The results obtained from 12 rabbits so tested showed again that spreading caused enhancement of the lesions at all dilutions of the bacteria.

As pointed out in Table I pustules resembling those produced by certain filterable viruses and by certain bacteria were observed. In section these proved to be small tubercles, about 2 mm. in diameter. They appeared when small quantities of tubercle bacilli were spread through the skin. As shown in the next section small quantities of dead bacteria, when spread, also produce such lesions.

Experiments with Heat-Killed and Defatted Bacilli.—The preceding tests with various quantities of bacilli were repeated using suspensions of bacilli which had been killed by heating in a water bath at 70°C. for 1 hour, and with bovine tubercle bacilli which had been subjected to extraction with an alcohol-ether mixture for 2 months. As in the previous tests each strain was injected with and without testicle extract in the same rabbit, each test save one being repeated 2 or 3 times. Results based on 14 rabbits are given in Table II.

It is clear from Table II that the lesions resulting from the injection of heat-killed bovine (Ravenel), Human H 37, and B.C.G. were considerably smaller than the lesions produced by like amounts of living bacilli, while on the other hand the bovine (B-1) strain and the Avian 1 strain were little affected by heating so far as their ability to produce skin lesions was concerned. In all cases, save the Human H 37 and the Ravenel strain, there was a definite enhancement as a result of spreading with testicle extract. In the case of the 2 strains excepted, the lesions induced were so small that enhancement was doubtful or

TABLE I
Average Size of Lesions Produced by Spreading Decreasing Quantities of Tubercle Bacilli in the Skin of Normal Rabbits
(Recorded at the Time of Maximum Lesion)

Strain injected	Amount of culture injected												Time of earliest clear lesion	Time of maximum lesion	No. of tests
	0.5 mg.		0.05 mg.		0.005 mg.		0.0005 mg.		0.00005 mg.		0.000005 mg.				
	Saline	Testicle extract	Saline	Testicle extract	Saline	Testicle extract	Saline	Testicle extract	Saline	Testicle extract	Saline	Testicle extract			
	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	days	days	
Bovine (B-1)	3.6	29.7	0.4	9.1	0.4	18.8							2-5	28-30	3
Bovine (Ravenel)	2.1	16.5	0.4	21.3 _p	0.3	1.5							10	30-32	2
Human (H 37)*	5.0	18.9	2.9	20.0	0.9 _e	1.9 _e	0.9 _e	0.3 _e	0.3	0.1	0.1	0.1	10	16-20	5
Avian S.	5.0	25.1	2.5	17.6	2.0	7.2	0.0	0.1	0.0	0.0	0.0	0.0	2-5	15	3
B. C. G.	4.8	17.0	2.2	9.2	1.8	3.2 _p	0.9	0.8 _p	0.5	0.4	0.0	0.0	2-5	15	2

p = pustular lesion.

e = erythematous lesion.

* The last three dilutions of the strain were each tested in a single rabbit.

TABLE II
Average Size of Lesions Produced by Spreading Decreasing Quantities of Heated and Defatted Tubercle Bacilli in Normal Rabbits
(Recorded at the Time of the Maximum Lesion)

Strain injected	Amount of culture injected										Time of earliest clear lesion	Time of maximum lesion	No. of tests
	0.5 mg.		0.05 mg.		0.005 mg.		0.0005 mg.		0.00005 mg.				
	Saline	Testicle extract	Saline	Testicle extract	Saline	Testicle extract	Saline	Testicle extract	Saline	Testicle extract			
	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	days	days	
Heated Bovine B-1	3.5	24.9	1.8	5.0	0.8	1.3	0.2	0.1	0.0	0.0	4-9	28-30	3
Heated Bovine Ravenel		2.0	±	±	±	±	0.0	0.0	0.0	0.0		30	1
Heated Human H 37	1.0	1.0	0.5	0.5 _p	±	±	0.0	0.0	0.0	0.0		16-20	3
Heated Avian S.	8.2	22.1	1.3	15.8	0.4	11.2	0.4	9.3	0.2	18.8	2-5	15	3
Heated B. C. G.	1.8	4.2	1.0	1.5	0.8	0.8	0.3	0.5	0.0	0.1	2-5	15	2
Defatted Bovine B-1	2.5	5.3	1.7	0.7	0.4	0.3	0.4	0.3	0.3	0.0	2	30	2

difficult to estimate.¹ It is also clear that the process of defatting to which the bovine bacilli were subjected rendered them much less capable of inducing severe lesions than either killed or living bacilli. When large quantities of defatted bacilli were spread, enhanced lesions resulted, but when smaller quantities were injected with testicle extract, partial suppression of the lesions occurred.

It would appear from this group of experiments that the tubercle bacilli behave somewhat like the filterable viruses and also like certain virulent bacteria such as Pneumococcus Type I, in that when they are injected together with testicle extract, it is not possible to demonstrate any suppression of lesions even when the smallest quantities are injected. This property is retained after exposure to 70°C. for 1 hour. In the case of defatted bacilli, the removal of the lipoids apparently made possible the suppression of lesions after the injection of small quantities with testicle extract. In this sense the defatted bacilli behaved like the non-acid fast bacteria.²

*The Effect of Testicle Extract on Experimental Tuberculosis in
Guinea Pigs*

Some of the foregoing experiments were repeated using guinea pigs instead of rabbits.

6 guinea pigs were injected intradermally in the flank with a suspension of 0.1 mg. of human tubercle bacilli (H 37) in 2.0 cc. of testicle extract. 5 other guinea pigs were similarly injected, using saline solution instead of testicle extract.

5 guinea pigs were injected with a similar amount of bacilli suspended in testicle extract, subcutaneously in the right groin, and 9 controls were injected with the same dose suspended in saline solution.

¹ The explanation of why certain strains of acid-fast bacilli lose more of their ability to produce lesions after being killed by heat than do others awaits further study, which is now being carried on.

² As a further illustration of the importance of lipoids in the genesis of lesions after the bacteria have been dispersed, the following results of some experiments with *M. phlei* seem worthy of record. This acid-fast bacillus is completely avirulent for laboratory animals, and induces only very mild lesions after injection into the skin of rabbits. Such lesions, had they been produced by an avirulent non-acid-fast strain, would have been most easily suppressed by the spreading action of testicle extract. However, following the injection of several dilutions of the strain *M. phlei*, both living and after heating at 70°C. for 1 hour, there was a uniform enhancement of all lesions as the result of spreading.

In both groups of guinea pigs the addition of testicle extract caused a marked enhancement of the primary lesion; those inoculated in the skin of the flank developed large shallow ulcers, about 30 to 35 sq. cm. These lesions are illustrated in Figs. 4, 5, and 6. In those animals inoculated in the groin, extensive lesions occurred which involved the entire hypogastrium and extended down the right thigh. Marked induration of the tissues occurred, with splinting of the entire hind quarter. Ulceration and drainage of purulent material occurred at several scattered points. The control animals, injected with saline solution suspension developed typical small nodules at the point of

TABLE III

The Extent of the Generalized Lesions Found in Guinea Pigs Inoculated with Tubercle Bacilli Suspended in Testicle Extract and Saline Solution Respectively

		No. of animals	Average weight		Average involvement		Time of autopsy days
			Spleen gm.	Lymph nodes gm.	Lungs	Liver	
Group I, intradermal injection	Testicle extract	6	6.6	7.2	+++±	+++	48
	Saline solution	5	3.8	7.7	+++	+++	
Group II, subcutaneous injection	Testicle extract	5	2.5		+++	+++	60
	Saline solution	9	1.5		++	+	

injection, which ulcerated and drained after the 2nd week of infection.

The animals treated intradermally were sacrificed 48 days after injection and the ones inoculated in the groin were sacrificed after 60 days. The extent of the involvement was recorded and these data are shown in Table III.

It will be seen from Table III that the spreading of the injected bacilli in the skin resulted in a more widespread general disease, especially in the spleen and lungs. This study of the effect of testicle extract on the primary infection of guinea pigs confirms the findings in similar studies on rabbits.

The Effect of Testicle Extract on the Tuberculin Reaction

It has been shown (4) that the dispersion of antigens in the skin of a sensitized animal by means of testicle extract results in a marked increase in the size of the ensuing (Arthus or Shwartzman) reaction, which is attended by a corresponding diminution of the severity of the reaction, which may even be completely suppressed. With this in mind, it was decided to study the effects of dispersion of the antigen involved in the production of the tuberculin reaction. At the same time a study was made of the effects of such a modified reaction upon the evolution of the disease.

The following experiments were performed to study the result of adding testicle extract to the tuberculin used in making skin tests in guinea pigs.

5 guinea pigs which had been infected 30 days previously by the subcutaneous injection of 0.1 mg. of human tubercle bacilli (Strain H 37) were inoculated intradermally with mixtures of tuberculo-protein MA 100 (Sharpe and Dohme) and testicle extract. 5 tuberculous guinea pigs were injected with mixtures of tuberculo-protein and salt solution as a control. Each animal was given 1 injection; the amount of tuberculo-protein given was varied from 0.25 mg. to 3.0 mg. This was mixed with 0.7 cc. of testicle extract or 0.7 cc. of saline solution.

At the end of 24 hours the 5 control animals showed strong reactions to the tuberculin, which in the following days became necrotic. In the 5 injected with the mixtures of tuberculo-protein and testicle extract the reactions at the end of 24 hours were diffuse, covering about 20 sq. cm., and showed only a reddening of the skin. These reactions faded rapidly, and at the end of 48 to 72 hours had disappeared with no ensuing necrosis.

A second experiment was performed to determine whether or not the diminution of the intensity of the skin reaction by spreading had any effect upon the essentially toxic action of large doses of tuberculin on tuberculous guinea pigs.

5 tuberculous guinea pigs were injected with 10 mg. of tuberculo-protein, mixed with 1.0 cc. of testicle extract. 5 control tuberculous guinea pigs were injected with 10 mg. of tuberculo-protein mixed with 1.0 cc. of salt solution.

The testicle extract caused dispersion of the tuberculin, as evidenced by the diffuse nature of the reaction, and the reduction of the intensity

of the local reaction per unit area of skin. The symptoms of tuberculin intoxication were in no way reduced by such dispersion; the animals became sick and weak, and several in each group died during the following 14 days. The temperature reaction of both groups of animals to the injections were similar; a rise of 1.5 degrees during the first 6 hours being followed by a return to normal limits.

From these experiments it appears that, although the spreading of tuberculin through the skin of a tuberculous guinea pig results in a reduction in the intensity of the lesion per unit area of tissue, the systemic effects are in no way altered.

The Effect of Testicle Extract on the Koch Phenomenon in Rabbits

The reaction of a tuberculous animal to a second infection is characterized by an acute inflammatory response, with a tendency to localization and destruction of the injected bacilli. The following experiment was performed in order to study the local and general reactions of tuberculous rabbits to the injection of tubercle bacilli dispersed through the skin.

Experiments with Living Bacilli.—15 rabbits which had been infected by the intravenous injection of 0.5 mg. of bovine tubercle bacilli Strain B-1, 35 days previously, were divided into 3 groups of 5. One group was injected in the skin with 1.0 mg. of B-1 bacilli suspended in 1.0 cc. of testicle extract; another group was similarly injected with a suspension made in saline solution; and the third group left as controls. 3 previously normal rabbits were injected intradermally with the suspension in testicle extract, and 3 more with the saline suspension, as controls of the viability of the organisms and for comparison of the lesions of primary infection with those of superinfection.

In the tuberculous rabbits there was a rapid response to the injection of bacilli, coming to a maximum within 48 hours. In the animals injected with testicle extract the lesions were very large, averaging 55 sq. cm., in contrast to the animals injected with saline suspensions, which showed small abscesses in the skin averaging about 3 sq. cm. These latter lesions ulcerated and drained purulent material after the 6th to 8th day. After the 2nd week they showed a reduction in size and showed a tendency to heal, which was complete at 6 weeks.

The lesions in the group superinfected with bacilli mixed with testicle extract were large, indurated, raised, and sharply circumscribed.

During the first 2 weeks they became increasingly indurated, although showing less evidence of inflammation; in the subsequent weeks a very slow regression took place, the lesions becoming smaller and less indurated. 6 weeks after injection the lesions were practically healed, with slight scarring of the skin in some instances. Microscopic sections of the skin taken at autopsy showed a layer of epithelioid cells lying just beneath the epithelium. This is illustrated in Fig. 7.

The previously normal animals injected at the same time, with a similar dose of bacilli in testicle extract, developed large ulcerated lesions such as those already described, which drained purulent material for some time and which at the end of 6 weeks were but partially closed.

The entire group of animals were sacrificed 50 days after their injection and it was found that the tuberculous animals which had been superinfected did not show any more visceral lesions than the animals which had received their primary infection at the same time but which were not superinfected. All showed a moderate amount of tuberculosis, limited to the lungs. The 3 previously normal animals which were inoculated with the same suspension with testicle extract showed more advanced pulmonary tuberculosis than in the corresponding animals inoculated with saline suspension of bacilli, and more than either of the groups subjected to superinfection. There was also involvement of the spleen in one case and of the testicles in another case.

The use of testicle extract brought out another striking difference between the reaction of normal and tuberculous rabbits to an inoculation with tubercle bacilli. While the previously normal animals developed large lesions which became ulcerated, the lesions of the tuberculous animals did not undergo ulceration, but simply regressed and healed after reaching a maximum size and intensity.

Experiments with Dead Bacilli.—15 rabbits, infected by the intravenous injection of 0.1 mg. of tubercle bacilli (Ravenel) were divided into 3 groups of 5. 47 days after inoculation one group was injected intradermally with 0.1 mg. of killed Ravenel culture, suspended in 2.0 cc. of testicle extract. Another group was similarly injected with killed bacilli suspended in saline solution, and the third group was left as a control. 8 days later the injections were repeated.

As in the foregoing experiment with living bacilli there was a rapid response to the injection, and as usual, the lesions produced by the injection of bacilli with testicle extract were several times larger than those in the controls.

The rapidly progressive disease caused by infection with the Ravenel strain manifested itself in the death of 7 out of 15 animals with far advanced tuberculosis within the next 40 days—a total of 95 days after the original infection. At this date the remainder of the animals was sacrificed. Of the 7 animals that died before this time, 4 were in the group reinjected with bacilli in saline solution, 2 were in the untreated control group, and only 1 in the group injected with the bacilli suspended in testicle extract.

In Table IV the autopsy findings are expressed as an average for the 3 groups.

It is evident from Table IV that the rabbits injected with the bacilli suspended in testicle extract showed less tuberculosis than the group injected with the saline suspension, and also less than the control group. In summarizing, it is evident that the addition of testicle extract to a suspension of tubercle bacilli, living or dead, injected into a tuberculous rabbit, resulted in a marked increase in the size of the local lesion, but without any corresponding diminution of the severity, as was the case in the Arthus, Shwartzman, and tuberculin reactions. The tuberculous rabbits, however, were not only able to resist further infection of the viscera following such injections, but in the case of the last experiment showed a considerable reduction in the extent of the visceral lesions.³

The Effect of Spreading Progressively Smaller Quantities of Tubercle Bacilli in the Skin of Tuberculous and Immunized Rabbits

The same procedure of spreading progressively smaller quantities of bacilli described in the experiments with normal rabbits was employed in tuberculous rabbits and rabbits that had been immunized by repeated injections of heat-killed tubercle bacilli.

The animals were prepared as follows: A group of 5 rabbits were given a total of 12 injections of heat-killed Avian 1 bacilli, over a period of 3 months. 1.0 mg.

³ Some additional experiments have shown that intradermal injection of 0.5 mg. of heat-killed Avian 1 bacilli with and without testicle extract into a rabbit prepared by successive injections of heat-killed bacilli of the same strain, resulted in lesions which had the same character as far as size, severity, and evolution were concerned, as those which were produced by the living bacilli.

was given at each dose. The injections were given both intravenously and intradermally, alternately.

Another group of 2 rabbits had been inoculated intravenously with 1.0 mg. of Bovine tubercle bacilli (B-1) 2 months previously. Both groups of animals were injected with progressive dilutions of the corresponding strain of living tubercle bacilli, mixed with testicle extract. Similar injections were made on the opposite flanks, using suspensions in saline solution. The results are expressed in Table V.

It is clear from Table V that spreading enhances the skin lesions resulting from such injections, at all dilutions, in tuberculous rabbits as well as in the immunized ones.

The Effect of Testicle Extract on the Koch Phenomenon in Guinea Pigs

When tuberculous guinea pigs are reinoculated intracutaneously with tubercle bacilli, the response of the animals to such an injection is characterized by a rapidly developing acute inflammatory reaction. Extension of the infection to the regional lymph glands is prevented or delayed (11), and healing of the local lesion occurs. This state of resistance acquired as a result of tuberculous infection is not complete; as Kraus and others have shown, using animals infected with a strain of tubercle bacilli incapable of producing progressive disease in guinea pigs. Such animals when superinfected with a virulent strain, eventually develop progressive tuberculosis, although far outliving previously normal animals injected with a similar dose.

In the foregoing section we have shown that the addition of testicle extract to the superinfecting dose of bacilli in rabbits does not increase the extent of the visceral lesions, as compared with animals similarly superinfected with bacilli suspended in saline solution. These experiments were repeated on a larger scale using tuberculous guinea pigs to study the effects of such superinfection on their resistance to the disease. A total of 93 guinea pigs were used in five consecutive experiments. The following is a typical protocol.

A group of 30 guinea pigs were inoculated with 0.01 mg. of living human tubercle bacilli, Strain H 37. The injection was made in the right groin. 6 weeks later 10 of these animals were inoculated with a suspension of 0.1 mg. of the same Strain H 37, in testicle extract, the injection being made into the skin of the flank. 10 additional animals of the original 30 were reinoculated with a like dose of bacilli, suspended in saline solution, the remaining 10 being left as controls of the primary infection. The resulting lesions were observed daily, and drawings and measurements of them were made. 6 weeks after superinfection the entire group was sacrificed and autopsies performed.

The reaction in the skin following the superinfection with organisms suspended in testicle extract was greatly enhanced. During the first 48 hours there was an acute inflammation of the skin, with hemorrhage and purple discoloration. The area involved was much greater than in the controls, approximately 15 times, but the intensity of the signs of inflammation during the first 48 hours was about the same.

The controls (superinfected with organisms suspended in saline) developed small nodules in the skin which, after the 4th day, broke down and drained purulent material. At the end of 2 weeks these started to show healing. By this time the lesions resulting from the superinfection with testicle extract had undergone necrosis, with extensive sloughing of the affected skin. The resulting ulcers healed only after many weeks.

Sections taken from such lesions 10 days after superinfection showed that the dispersion of the injected bacilli through the dermis had resulted in the formation of a thin layer of epithelioid cells lying in the collagenous portion of the skin. There was very little caseation, and none of the cellular reaction extended beneath the panniculus carnosus muscle. Sections stained with carbol-fuchsin showed the presence of very few tubercle bacilli, in contrast to the great number found in the sections from the control animals. The lesions in the controls were small abscesses, extending into the subcutaneous connective tissue, with a definite limiting zone of connective tissue around them. They were filled with caseous debris.

When the extent of the tuberculosis in the 2 groups was compared, it was found that the animals superinfected with the addition of testicle extract showed less tuberculosis than the animals superinfected with a saline suspension. The spleens were smaller, the extent of hepatic and pulmonary tuberculosis was diminished, and lymphatic involvement was not so marked. These results and those of the additional experiments are given in Table VI.

From Table VI it is seen that there was no apparent difference between the control group and the group superinfected with the saline suspension. Other workers (12) have stated that superinfection, under the proper conditions, has the effect of stimulating the resistance of the animals to the disease. It would seem evident that the conditions of time and dosage in our animals did not allow this effect

TABLE IV

The Extent of the Lesions in Rabbits Injected with Heat-Killed Tubercle Bacilli Suspended in Testicle Extract and Saline Solution Respectively 47 and 55 Days after Infection

	No. of animals	Average weight		Average involvement		
		Spleen	Lymph nodes	Lungs	Kidney	Bone marrow
		gm.	gm.			
Injected with bacilli suspended in testicle extract.....	5	2.7	1.5	+++	++±	+±
Injected with bacilli suspended in saline solution.....	5	2.9	2.0	++++	++++	++++
Untreated controls of primary infection.....	5	2.9	3.0	++++	++++	++±

TABLE V

Average Size of Lesions Produced by Spreading Decreasing Quantities of Tubercle Bacilli in the Skin of Immunized Rabbits (Recorded 15 Days after Infection)

Strain injected	Amount of culture injected												No. of tests	Method of preparation of the animals
	0.5 mg.		0.05 mg.		0.005 mg.		0.0005 mg.		0.00005 mg.		0.000005 mg.			
	Saline	Testicle extract	Saline	Testicle extract	Saline	Testicle extract	Saline	Testicle extract	Saline	Testicle extract	Saline	Testicle extract		
Avian S.....	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	sq. cm.	5	Vaccination
Bovine B-1.....	2.3	14.1	4.6	5.0	1.2	1.2	0.2	0.3	0.1	0.3	0.1	0.5	2	Infection

TABLE VI

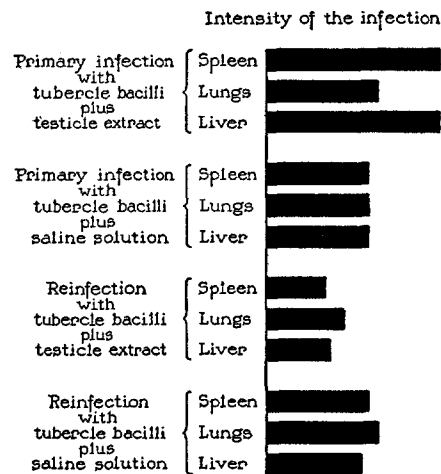
The Extent of Lesions Found in Guinea Pigs after Superinfection with Tubercle Bacilli Injected with Testicle Extract and Saline Solution Respectively (Recorded 6 Weeks after Superinfection and 12 Weeks after Primary Infection)

	No. of animals	Average weight		Average involvement	
		Spleen	Lymph nodes	Lungs	Liver
		gm.	gm.		
Testicle extract.....	31	3.5	5.4	++±	+±
Saline solution.....	36	6.0	7.4*	+++	+++
Controls of primary infection.....	26	6.1	6.2†	+++	+++

* Only 23 animals recorded.

† Only 17 animals recorded.

to be demonstrated, as long as the superinfection was made in the usual manner; but that the dispersion of the bacilli did in some fashion swing the balance in favor of the animals' resistance. It would derive perhaps that the rapid and acute inflammatory reaction tended to isolate and discharge the injected bacilli, so that actually very little absorption of bacillary material took place, and that dispersion of the bacilli, by increasing the intimacy with which they came into contact with the tissues and body fluids, increased the absorption of bacillary products. This property of a dispersing agent, of amplifying and accentuating reactions already determined qualitatively,



TEXT-FIG. 1

is especially brought out in the study of tuberculosis, in which a number of complex reactions may occur, one perhaps masking the others.

Text-fig. 1 gives a graphic representation of the differences in the extent of the disease which result from the injection of the tubercle bacilli with and without testicle extract in both normal and tuberculous guinea pigs.

The Effect of Parenteral Injections of Testicle Extract on Tuberculous Guinea Pigs

From the preceding experiments it is seen that the injection of testicle extract together with tubercle bacilli in the normal or tuber-

culous animal results in an enhancement of the local reaction to the injected bacilli. In the previously normal animal this is followed by an increase in the rate of development of visceral lesions, while in the tuberculous guinea pig, the effect is just the opposite. It was decided to test the effect of injecting large amounts of testicle extract parenterally into guinea pigs recently rendered tuberculous.

10 guinea pigs which had been infected with 0.10 mg. of human type H 37 bacilli 10 days prior, were divided into 2 groups of 5 each, one of which was given a series of injections of testicle extract subcutaneously. 5 doses were given during the following 19 days, starting with 2.0 cc. and increasing the dose up to 5.0 cc. A total of 19 cc. was given each animal. 6 days after the last injection the animals were sacrificed for autopsy. The extent of the tuberculosis in the 2 groups is plotted in Table VII.

It is seen from Table VII that there was no striking difference between the 2 groups, and no evidence was obtained that the administration of the testicle extract had either increased the rate of progress of the disease or changed the distribution of the lesions. On the contrary, what slight difference existed was in favor of the treated animals. The introduction of large amounts of the spreading agent into the tuberculous animals had no untoward effect, even in an infection of recent origin (10 days).⁴

The Effect of Spreading of the Antigen upon Resistance Produced by Preventive Vaccination of Guinea Pigs and Rabbits against Tuberculosis

The foregoing experiments, showing the favorable influence that dispersion of the reinfesting bacilli had on the course of the disease, led us to a study devised to increase the immunity conferred upon animals to tuberculosis by repeated injections of heat-killed vaccines.

⁴ As a complement to these experiments the following observations are worthy of record. In 3 rabbits with extensive tuberculous lesions in the skin, injections of testicle extract were made into the center and around the edges of the lesions. In no case was there any spread of the infection, and in 2 cases it seemed that a slight acceleration of healing took place.

Hanger (13) has reported that similar treatment of lesions induced by *Bacterium leprosepticum* resulted in a new spread of the infection.

Experiments on Guinea Pigs.—A total of 57 guinea pigs were used. 20 guinea pigs were vaccinated with a suspension of heat-killed human tubercle bacilli (H 37) mixed with testicle extract. 19 guinea pigs were vaccinated with a similar suspension in saline solution. A total of 5 injections were given at weekly intervals, into the skin of the flank. In the case of the testicle extract suspensions, the total amount of vaccine was not injected into one area, but divided into 2 portions, one of which was injected into the skin on each side. The first dose was 0.1 mg., the second was 0.2 mg., and the next 3 were 2.0 mg. each suspended in 2.0 cc. of testicle extract and 2.0 cc. of saline solution respectively.

18 days after the last injection of vaccine both groups were given a test inoculation of 0.1 mg. of living human tubercle bacilli (H 37) injected subcutaneously in the right groin. At the same time 18 previously normal guinea pigs were infected with the same amount of bacilli.

50 days after the test infection the entire group of animals was sacrificed and the extent of their visceral lesions compared. The results are recorded in Table VIII.

From Table VIII it will be seen that the extent of the disease in each of the vaccinated groups was less than that in the unvaccinated controls. It is also clear that the animals vaccinated with the testicle extract-vaccine mixture showed much less tuberculosis than either of the other 2 groups.

Experiments on Rabbits.—14 rabbits were used. 5 rabbits were given 4 injections of 2.0 mg. of heat-killed bovine tubercle bacilli (Ravenel) mixed with 2.0 cc. of testicle extract, at weekly intervals. The vaccine-testicle extract mixture was divided into 2 portions, and injected into both flanks. 5 rabbits were similarly vaccinated with 2.0 mg. of heat-killed Ravenel, suspended in saline solution. These injections were made in one area only.

17 days after the last injection of vaccine the 10 rabbits together with 4 previously normal rabbits were given a test inoculation of 0.1 mg. of living Ravenel bacilli, injected intradermally in the interscapular region.

Observations of these animals during the next few days disclosed an interesting fact, that the local lesions resulting from the intradermal infection in the group that had been vaccinated with the vaccine-testicle extract mixture were definitely larger, more inflamed, and more indurated than those found in the animals vaccinated with the organisms suspended in saline; these latter were in turn larger and more inflammatory than the lesions produced in the previously normal animals.

It was also observed that in the controls, and in the group vaccinated with the saline suspension the axillary lymph nodes increased

TABLE VII

Effect of Repeated Injections of Testicle Extract on the Visceral Lesions of Tuberculous Guinea Pigs as Compared with Non-Injected Controls (Recorded 35 Days after Infection)

	No. of animals	Average weight		Average involvement	
		Spleen	Lymph nodes	Lungs	Liver
		gm.	gm.		
Treated with testicle extract.....	5	2.3	4.0	+	++±
Untreated controls.....	5	3.0	5.2	+±	++±

TABLE VIII

Extent of Involvement Found in Guinea Pigs Injected with Heat-Killed Tubercle Bacilli Plus Testicle Extract and Saline Solution Respectively, Prior to Their Infection with Tuberculosis (Recorded 50 Days after Infection)

	No. of animals	Average weight		Average involvement	
		Spleen	Lymph nodes	Lungs	Liver
		gm.	gm.		
Vaccine suspended in testicle extract.....	20	3.5	4.6	+±	+±
Vaccine suspended in saline solution.....	19	4.43	5.7	++	++
Untreated controls of primary infection.....	18	5.1	5.0	++±	++±

TABLE IX

The Extent of Involvement Found in Rabbits Injected with Heat-Killed Bovine Tubercle Bacilli Plus Testicle Extract and Saline Solution Respectively, Prior to Their Infection with Tuberculosis (Recorded 81 Days after Infection)

	Rabbit No.	Right axillary lymph node	Left axillary lymph node	Lungs	Kidneys	Intestines
Vaccine suspended in testicle extract	5i	0	0	++	++	0
	6i	0	0	+	0	0
	7i	0	0	+	0	0
	8i	0	0	0	0	0
	9i	+	+	0	0	0
Vaccine suspended in saline solution	10i	++++	++++	+	0	0
	11i	0	+++	0	0	0
	12i	++++	++++	+	0	+
	13i	++++	++++	++	0	0
	14i	+++	+++	0	0	0
Untreated controls primary infection	75i	0	++++	++	0	0
	76i	++++	+++	+++	0	0
	77i	++++	+	++	0	+
	78i	++++	++++	++	+	+

in size and after 2 weeks became easily palpable. The animals in the group vaccinated with the testicle extract-vaccine mixture showed no palpable enlargement of the regional lymph nodes during the entire period of observation, save in one animal, which had a very slight enlargement. These observations were confirmed at autopsy, the entire group of animals being sacrificed 81 days after the test infection. The results of the autopsies are plotted in Table IX.

It is seen in Table IX that the extent of the disease in each of the vaccinated groups was less than in the unvaccinated controls. It is also clear that the animals vaccinated with the suspensions in testicle extract had considerably less involvement than either of the other 2 groups.

From the two experiments it is concluded that the dispersion of killed tubercle bacilli through the tissues markedly enhanced the degree of immunity conferred, when compared with animals injected with a like number of bacilli suspended in saline solution.

DISCUSSION

It has been shown previously with many strains of bacteria that the response of animal hosts to a given experimental infection is very largely affected by the degree of dispersion of the infectious agent through the tissues at the time of inoculation.

Studies have now been made of the effect of dispersion by means of testicle extract on the response of laboratory animals to experimental infection with several strains of tubercle bacilli. Dermal infections were found most suitable, as the extent of dispersion could be readily measured. It has been shown that the spreading of bacilli injected into the skin results in an enhancement of the local lesion, whether the number of bacilli injected was large or small. Enhancement of the local lesion also occurred irrespective of the strain of tubercle bacilli employed. In the case of bacilli virulent for the animal used, such enhancement of the local lesion was accompanied by an acceleration in the rate at which visceral lesions developed. When strains non-virulent for the animal were injected, no extension to the viscera occurred, despite the development of a large local lesion.

The findings emphasize the fact that owing to their peculiar chemical constitution the tubercle bacilli are uniformly capable of producing

the characteristic lesions when introduced into the tissues, entirely irrespective of their ability to invade the tissues of the particular host chosen. It would seem that this accounts for the fact that quantities of bacilli which approach the minimal infective dose are enhanced by dispersion, in contrast to other non-acid-fast bacteria (as for instance the staphylococcus), which may fail entirely to produce a lesion when small quantities are dispersed through the skin. The fact that enhancement occurs similarly with tubercle bacilli killed by heating further emphasizes this point.

In discussing the response of tuberculous animals to superinfection it is necessary to review briefly the reactions to superinfection as such. They are characterized by the rapid localization and destruction of the bacilli, processes which tend to prevent extension of the new process. Under certain conditions, furthermore, superinfection results in a stimulation of the general defense mechanism, resulting in an increased resistance to the already established disease.

Our studies showed that the dispersion of the bacilli employed in superinfection results in an enhancement of the local reaction to the bacilli. The signs of inflammation were more intense, and the lesion was increased greatly in size. In guinea pigs necrosis of the skin occurred, followed by superficial sloughing. The animals in which the bacilli were injected in salt solution developed small abscesses which quickly discharged their contents. Thus both groups of animals demonstrated that at the time of reinfection they were partially immune, as judged by the exhibition of the Koch phenomenon. 6 weeks after superinfection when all were sacrificed and autopsies performed it was found that the animals which had been injected with bacilli mixed with testicle extract showed much less visceral tuberculosis than those receiving the saline suspension of bacilli. It would seem likely that this difference resulted from an increased absorption of bacillary materials, which served as a stimulus to the defense mechanisms of the body. The greatly increased opportunity for such absorption supplied by the dispersion of the bacilli through a large area of skin is obvious. The animals which were superinfected with bacilli suspended in saline solution showed no less tuberculosis than did the untreated controls of primary infection. From this it would seem that under the conditions of our experiments the factors con-

cerned with the localization and elimination of the bacilli in the Koch phenomenon may have acted so efficiently as to preclude the possibility of any good arising from the superinfection, except when the spreading factor was employed.

Studies on the effect of superinfection on the course of tuberculosis in rabbits were not carried out as extensively as in guinea pigs. However, one interesting observation was made which accentuates the difference in the response of these 2 animal species to tuberculous infection. When tuberculous guinea pigs were superinfected in the skin, the appearance and development of the lesions closely simulated a reaction to tuberculin. The inflammatory response was rapid, and went on to necrosis of the skin. There was evidence of existent immunity, since no extension of the second infection to the viscera took place. When tuberculous rabbits were superinfected in the skin, the resulting lesions developed rapidly, but did not resemble in any way a tuberculin reaction; no necrosis or ulceration followed, although a very rapid formation of epithelioid cells did occur. These lesions regressed and healed after reaching a maximum size. This was best shown when the bacilli were dispersed through the skin with testicle extract. Previously normal rabbits developed large lesions which ulcerated and persisted, for many weeks, after a similar infection.

It is well known that rabbits do not exhibit a high degree of skin hypersensitiveness to tuberculin, and yet they do demonstrate a marked resistance to superinfection. Moreover, the elimination of the injected tubercle bacilli by means of abscess formation or by casting off of a slough does not seem to be a necessary element in this resistance to superinfection. It is suggested that the reaction of tuberculous guinea pigs to superinfection is an immune reaction complicated by the presence of allergy, and that the allergy is of aid only as it results in the death and elimination of the infected tissue. In rabbits the reaction is clearly an immune reaction uncomplicated by the presence of allergy.

The vaccination of both rabbits and guinea pigs against tuberculosis with heat-killed cultures of tubercle bacilli was carried out, with and without the use of testicle extract as a dispersing agent. It was shown that the dispersion of the vaccine through the skin resulted in an increased resistance to a test inoculation with living, virulent tubercle bacilli.

The action of testicle extract as a dispersing agent in experimental tuberculosis may be likened to that of an amplifying device. The factors of host immunity and bacterial virulence determine the nature and direction of the response to infection, but the degree of dispersion of the bacilli in the tissues determines to a large extent the magnitude of the response.

SUMMARY

1. The skin lesions in rabbits and guinea pigs following intradermal injection of tubercle bacilli (5 strains) were greatly increased in size and severity when testicle extract was added to the inoculum. Such enhancement was followed by a more widespread and rapidly progressing disease only when virulent strains were employed.

2. Attempts to suppress the development of skin lesions resulting from the injection of either normal or tuberculous rabbits with very small quantities of tubercle bacilli mixed with testicle extract were unsuccessful.

3. The skin reactions of tuberculous guinea pigs to tuberculo-protein MA 100 were greatly increased in size and markedly reduced in intensity by the addition of testicle extract to the protein solution. The toxic effect of larger quantities of tuberculo-protein was not altered by the addition of testicle extract.

4. The dispersion of tubercle bacilli through the skin of tuberculous rabbits resulted in a marked enhancement of the Koch phenomenon but was not followed by any extension of the new infection to the viscera. Tuberculous rabbits injected on two occasions with dead tubercle bacilli suspended in testicle extract showed an increased resistance to the disease when compared with controls receiving dead bacilli suspended in saline solution.

5. The resistance conferred upon tuberculous guinea pigs by superinfection was greatly increased when the bacilli employed were dispersed through the skin with testicle extract.

6. The parenteral administration of large quantities of testicle extract to recently infected guinea pigs did not result in any increase in the extent of the visceral lesions.

7. The partial immunity conferred upon guinea pigs and rabbits by vaccination with heat-killed tubercle bacilli was increased as a result of dispersion of the vaccine through the skin with testicle extract.

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EXPLANATION OF PLATES

PLATE 1

FIG. 1. Rabbit 143-H. Primary infection, 16 days after inoculation with 1.0 mg. of bovine (B-1) bacilli, suspended in 2.0 cc. of saline solution.

FIG. 2. Rabbit 2-H. Primary infection, 16 days after inoculation with 1.0 mg. of bovine (B-1) bacilli, suspended in 2.0 cc. of testicle extract.

FIG. 3. Rabbit 52-H. Primary infection, 34 days after inoculation with 1.0 mg. of bovine (B-1) bacilli, suspended in 2.0 cc. of testicle extract.

PLATE 2

FIG. 4. Guinea Pigs 7-86 (upper) and 7-95 (lower). Primary infection. Guinea Pig 7-86 inoculated 36 days previously with 0.1 mg. of human (H 37) bacilli suspended in 2.0 cc. of testicle extract. Guinea Pig 7-95 was inoculated at the same time with 0.1 mg. suspended in 2.0 cc. saline solution.

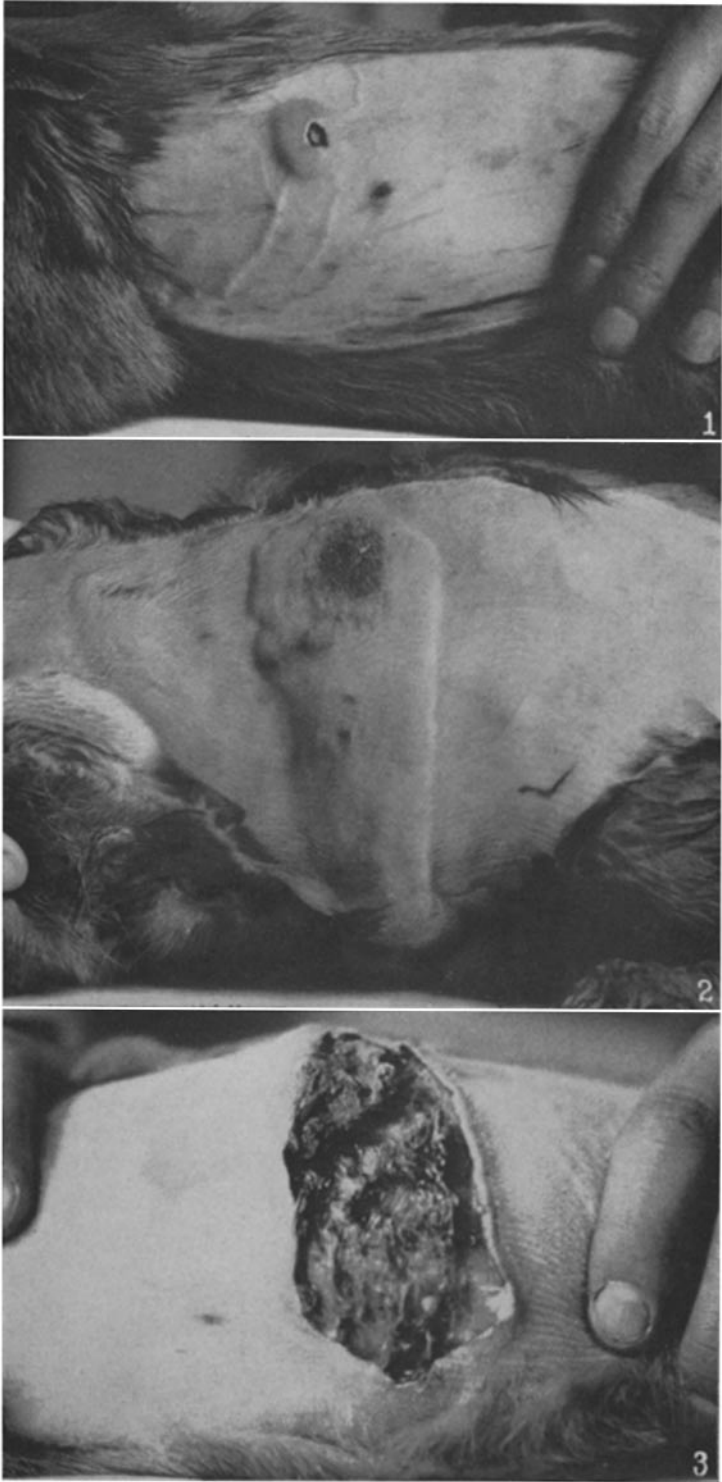
FIG. 5. Guinea Pigs 22-H and 25-H, 4 days after superinfection with 0.1 mg. of human (H 37) bacilli. Guinea Pig 25-H (upper) received bacilli suspended in saline solution; Guinea Pig 22-H (lower) received bacilli suspended in testicle extract. Arrows point to the limits of swelling.

FIG. 6. Guinea Pigs 18-H and 14-H, 18 days after superinfection with 0.1 mg. of human (H 37) bacilli. Guinea Pig 18-H (upper) received bacilli suspended in saline solution. Guinea Pig 14-H (lower) received bacilli suspended in testicle extract.

PLATE 3

FIG. 7. Rabbit 55-H. Section of skin lesion taken 50 days after superinfection with bovine tubercle bacilli suspended in testicle extract. Arrow points to layer of epithelioid cells.

Stained with Masson's trichrome stain. Magnification $\times 50$. No color filter used. Light diffused through ground glass.



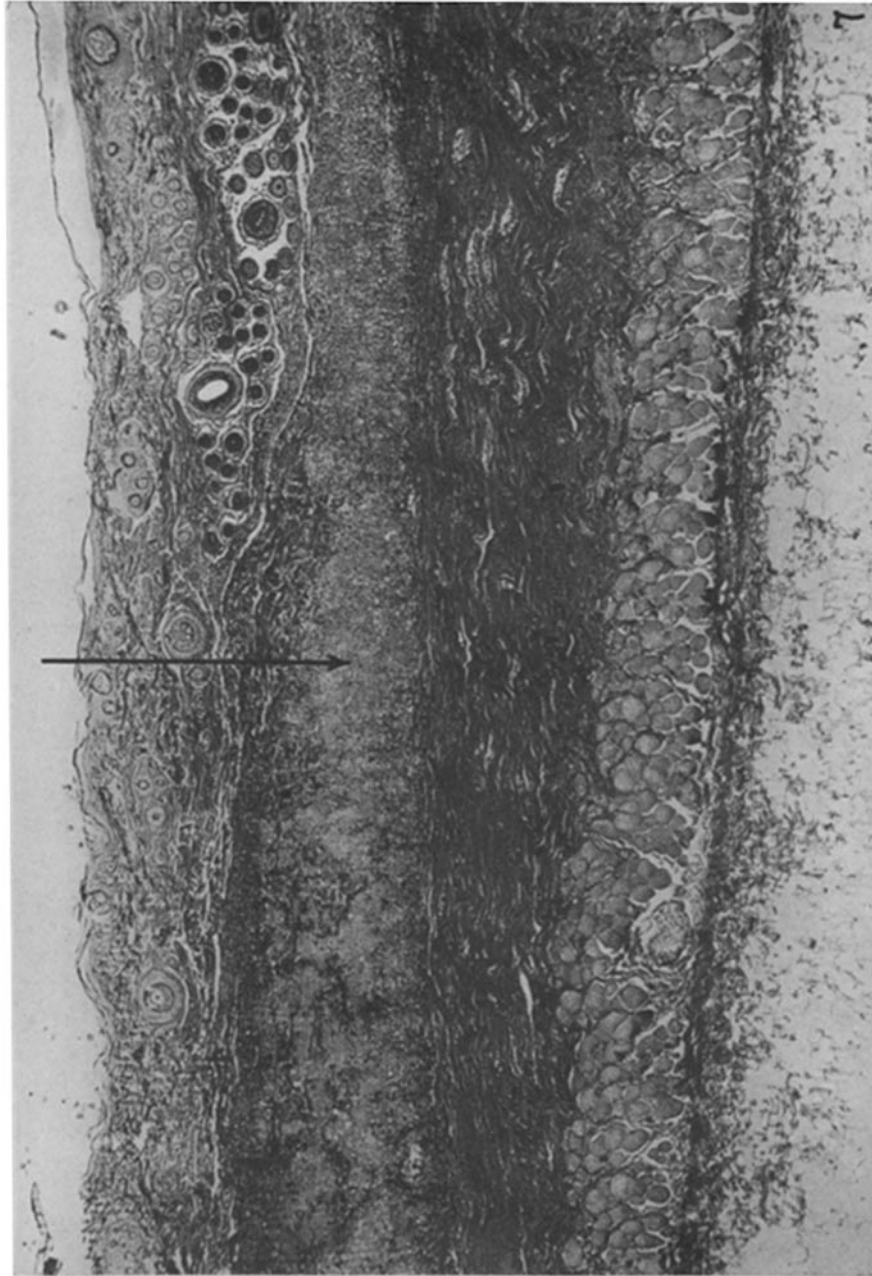
Photographed by Joseph B. Haulenbeek

(Thomas and Duran-Reynals: Dispersion of tubercle bacilli in infection)



Photographed by Joseph B. Haulenbeck

(Thomas and Duran-Reynals: Dispersion of tubercle bacilli in infection)



Photographed by Louis Schmidt

(Thomas and Duran-Reynals: Dispersion of tubercle bacilli in infection)